PTO/SB/80 (11-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:

[x] Practitioners associated with the Customer Number: 123223

OR

[ ] Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

[x] The address associated with Customer Number: 123223

OR

[ ] Firm or Individual Name

Address					
City		State		Zip	
Country		Telephone		Email	

Assignee Name and Address:

BASF Plant Science GmbH
67056 Ludwigshafen
Germany

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	25. APR. 2014
Name	Dr. Andreas Pohl / Dr. Uwe Prüßer	Telephone	
Title	Vice Presidents		

Docket No.: 074017-0013-01-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: Not Yet Assigned | Confirmation No.: N/A

Filed: Concurrently Herewith | Art Unit: N/A

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT (IDS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 CFR 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement accompanies the new continuation patent application submitted herewith and corresponds to the Information Disclosure Statements previously submitted or cited in parent Application Serial No. 12/280,090.

Copies of the references on the PTO/SB/08 are not provided herewith pursuant to 37 CFR § 1.98(d), because the references have been previously cited or submitted in parent Application Serial No. 12/280,090.

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this Information Disclosure statement shall not be construed to be an admission that any patent,

publication or other information referred to therein is "prior art" for this invention unless specifically designated as such. Moreover, Applicant understands that the Examiner will make an independent evaluation of the cited documents.

It is submitted that the Information Disclosure Statement is in compliance with 37 CFR 1.98 and the Examiner is respectfully requested to consider the listed references.

Applicant believes no fee is due with this submission. However, if a fee is due, the Director is hereby authorized to charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US, from which the undersigned is authorized to draw.

Respectfully submitted,

By___/ *Hui-Ju Wu* /_____

Hui-Ju Wu, Ph.D.
Registration No.: 57,209
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Attorney for Applicant

#85,080,132

PTO/AIA/15 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	074017-0013-01-US
First Named Inventor	Petra CIRPUS
Title	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 90]
   Both the claims and abstract must start on a new page.
   (See MPEP § 608.01(a) for information on the preferred arrangement)
5. [ ] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets*]
6. **Inventor's Oath or Declaration** [*Total Pages* 4]
   (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))
   a. [ ] Newly executed (original or copy)
   b. [X] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.*
   See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers**
    (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [X] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [X] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [X] **Other:** Text File of Sequence Listing Submitted *via* EFS Web

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 123223 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Hui-Ju Wu/	Date	September 6, 2016
Name (Print/Type)	Hui-Ju Wu, Ph.D.	Registration No. (Attorney/Agent)	57,209

#85,078,564

Application Data Sheet

Inventor Information

Inventor Number::	1
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Country of mailing address::	Canada
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Postal or Zip Code of mailing address::	S7N 3S5

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Inventor Number::	7
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City of mailing address::	Mannheim
Country of mailing address::	Germany
Postal or Zip Code of mailing address::	68163

Correspondence Information

Correspondence Customer Number::	123223

Application Information

Application Type::	Regular
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	Text File *via* EFS Web
Computer Readable Form (CRF)?::	See Text File *via* EFS Web
Title::	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
Attorney Docket Number::	074017-0013-01-US
Request for Early Publication?::	No
Request for Non-Publication?::	No
Small Entity?::	No
Petition included?::	No
Portions or all of the application associated with	No

this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013:: No

Representative Information

Representative Customer Number:: 123223

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Continuation of	12/280,090	08/20/08
12/280,090	National Stage of	PCT/EP2007/051675	02/21/07

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed::
European Patent Office	06120309.7	09/07/06	Yes
Germany	102006008030.0	02/21/06	Yes

Applicant Information

Applicant Number:: 1

Applicant Type:: Assignee

Organization Name:: BASF Plant Science GmbH

Street of mailing address:: Carl-Bosch-Str. 38

City of mailing address::	Ludwigshafen
Country of mailing address::	Germany
Postal or Zip Code of mailing address::	67056

Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Hui-Ju Wu/	Date (YYYY-MM-DD)	2016-09-06
Name	Hui-Ju Wu, Ph.D.	Registration Number	57,209

Declaration, Power of Attorney and Petition

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

the specification of which

[ ] is attached hereto.

[ ] was filed on _____ as

Application Serial No. _____

and amended on _____.

[x] was filed as PCT international application

Number _____

on _____

and was amended under PCT Article 19

on _____ (if applicable)

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a) - (d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International Application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed	
102006008030.0	Germany	21 February 2006	[x] Yes	[ ] No
06120309.7	Europe	07 September 2006	[x] Yes	[ ] No

pdaspect.rdf

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	**Filing Date**	**Status (pending, patented, abandoned)**

I hereby appoint the registered practitioner(s) associated with Customer No. **23416** to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Direct all correspondence to Customer Number **23416**.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Petra Cirpus
NAME OF SOLE OR FIRST INVENTOR

Signature of Inventor

Date 4.5.2007

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Citizen of Germany

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Jörg Bauer
NAME OF SECOND JOINT INVENTOR

Signature of Inventor

Date 04.05.2007

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Citizen of Germany

Post Office Address: same as residence

Xiao Qiu
NAME OF THIRD JOINT INVENTOR

Signature of Inventor

Date March 29, 2007

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Citizen of Canada

Post Office Address: same as residence

Guohai Wu
NAME OF FOURTH JOINT INVENTOR

Signature of Inventor

Date March 29, 2007

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Citizen of Canada

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Bifang Cheng
NAME OF FIFTH JOINT INVENTOR

Signature of Inventor

Date March 29, 2007

Residence:
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Canada
Citizen of Canada

Post Office Address: same as residence

---

pdusgot.rdf

Martin Truksa
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Canada
Citizen of Canada

Post Office Address: same as residence

X
Signature of Inventor

Date X April 12, 2007

Tom Wetjen
NAME OF SEVENTH JOINT INVENTOR

Residence:
Meerwiesenstrasse 21
68163 Mannheim
Germany
Citizen of Germany

Post Office Address: same as residence

Signature of Inventor

Date 4.5.2007

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PTO/SB/08b (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449A/B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	6

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA*	US-5,614,393	03-25-1997	Thomas T. L. et al.	
	AB*	US-6,043,411	03-28-2000	Nishizawa et al.	
	AC*	US-2004/0111763	06-10-2004	Heinz et al.	
	AD*	US-6,884,921-B2	04-26-2005	Browse et al.	
	AE*	US-6,459,018-B1	10-01-2002	Knutzon	
	AF*	US-7,550,286-B2	06-23-2009	Damude et al.	
	AG*	US-7,777,098-B2	08-17-2010	Cirpus et al.	
	AH*	US-2004/0172682-A1	09-02-2004	Kinney et al.	
	AI*	US-2004/0049805-A1	03-11-2004	Lerchl et al.	
	AJ*	US-2004/0053379-A1	03-18-2004	Lerchl et al.	
	AK*	US-2008/0155705-A1	06-26-2008	Zank et al.	
	AL*	US-2009/0222951-A1	09-03-2009	Cirpus et al.	
	AM*	US-2010/0021976-A1	01-28-2010	Lerchl et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	BA**	WO-91/13972	09-19-1991	Calgene Inc.		
	BB**	WO-93/06712	04-15-1993	Rhone-Poulenc Agrochimie		
	BC**	WO-93/11245	06-10-1993	E.I. DuPont De Nemours and Co.		
	BD**	EP-0 550 162	07-07-1993	Pioneer Hi-Bred International, Inc.		
	BE**	WO-94/11516	05-26-1994	E.I. duPont de Nemours And Company		
	BF**	WO-94/18337	08-18-1994	Monsanto Company & Michigan State University		
	BG**	WO-96/21022	07-11-1996	Rhone-Poulenc Agrochimie		
	BH**	WO-97/21340	06-19-1997	Cargill, Inc.		
	BI**	WO-97/30582	08-28-1997	Carnegie Institution Of Washington & Monsanto Co., Inc.		
	BJ**	WO-01/85968-A2	11-15-2001	Bioriginal Food & Science Corp.		
	BK**	EP-0 794 250	09-10-1997	Soremartec S.A. & Ferrero S.p.A.		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at **www.uspto.gov** or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

Substitute for form 1449A/B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 6

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	BL**	WO-98/46776	10-22-1998	Calgene LLC		
	BM**	WO-98/46764	10-22-1998	Calgene LLC & Abbott Laboratories		
	BN**	WO-98/46763	10-22-1998	Calgene LLC & Abbott Laboratories		
	BO**	WO-98/46765	10-22-1998	Calgene LLC & Abbott Laboratories		
	BP**	WO-99/27111	06-03-1999	University of Bristol		
	BQ**	WO-99/64616	12-16-1999	Abbott Laboratories		
	BR**	WO-00/12720	03-09-2000	Abbott Laboratories		
	BS**	WO-00/21557	04-20-2000	Merck & Co., Inc.		
	BT**	WO-01/59128	08-16-2001	BASF Aktiengesellschaft		See US 2004/0111763
	BU**	WO-02/08401	01-31-2002	Abbott Laboratories		
	BV**	WO-02/44320	06-06-2002	Xenon Genetics Inc.		
	BW**	WO-02/077213	10-03-2002	University of Bristol		
	BX**	CA-2 485 060	11-13-2003	BASF Plant Science GmbH		
	BY**	DE-102 19 203	11-13-2003	BASF Plant Science GmbH		See CA 2 485 060
	BZ**	WO-2004/071467	08-26-2004	E. I. duPont de Nemours and Company		
	BA1**	WO-02/057464-A2	07-25-2002	BASF Plant Science GmbH		See US2004/0049805
	BB1**	WO-03/064596-A2	08-07-2003	Abbott Laboratories		
	BC1**	WO-95/18222-A1	07-06-1995	Kirin Beer Kabushiki Kaisha		See US 6,043,411
	BD1**	WO-02/092540-A1	11-21-2002	Martek Biosciences Boulder Corporation		
	BE1**	WO-02/081668-A2	10-17-2002	Abbott Laboratories		
	BF1**	WO-2005/083093-A2	09-09-2005	BASF Plant Science GmbH		See US 2008/0155705 & US 2009/0222951
	BG1**	DE-101 02 337-A1	07-25-2002	BASF Plant Science GmbH		See US 2004/0053379 & US 2010/0021976
	BH1**	WO-2005/012316-A2	02-10-2005	BASF Plant Science GmbH		See Abstract
	BI1**	CA-2559360-A1	09-09-2005	BASF Plant Science GmbH		
	BJ1**	WO-2005/103253-A1	11-03-2005	Commonwealth Scientific And Industrial Research Organisation		

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Application Number	Not Yet Assigned
Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA**	"Danio rerio Polyunsaturated Fatty Acid Elongase Protein", GenBank Database Accession No. AAN77156, February 15, 2006.	
	CB**	Cronan, J.E. et al., "Biosynthesis of Membrane Lipids", in "E. coli und Salmonella", Section B2, Neidhardt, F.C. et al. eds., ASM Press, Washington, DC, (1996), pp. 612-636.	
	CC**	Gerhardt, B., "Fatty Acid Degradation in Plants", Prog. Lipid Res. 31:4 (1992), pp. 417-446.	
	CD**	Wada, H. et al., "Enhancement of Chilling Tolerance of a Cyanobacterium by Genetic Manipulation of Fatty Acid Desaturation", Nature 347 (1990), pp. 200-203.	
	CE**	Yu, R. et al., "Production of Eicosapentaenoic Acid by a Recombinant Marine Cyanobacterium, *Synechococcus* sp.", Lipids, 35:10 (2000), pp. 1061-1064.	
	CF**	Magnuson, K. et al., "Regulation of Fatty Acid Biosynthesis in *Escherichia coli*", Microbiological Reviews, 57:3 (1993), pp. 522-542.	
	CG**	Akimoto, M. et al., "Carbon Dioxide Fixation and Polyunsaturated Fatty Acid Production by the Red Alga *Porphyridium Cruentum*", Applied Biochemistry and Biotechnology 73 (1998), pp. 269-278.	
	CH**	Stymne, S., "Biosynthesis of 'Uncommon' Fatty Acids and Their Incorporation into Triacylglycerols", Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, N. Murata et al., Editors, The American Society of Plant Physiologists (1993), pp. 150-158.	
	CI**	Frentzen, M., "Acyltransferases from Basic Science to Modified Seed Oils", Fett/Lipid, 100:4-5, S. (1998), pp. 161-166.	
	CJ**	Shanklin, J. et al., "Desaturation and Related Modifications of Fatty Acids", Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998), pp. 611-641.	
	CK**	Drexler, H. et al., "Metabolic Engineering of Fatty Acids for Breeding of New Oilseed Crops: Strategies, Problems and First Results", J. Plant Physiol. 160 (2003), pp. 779-802.	
	CL**	Domergue, F. et al., "Cloning and Functional Characterization of *Phaeodactylum tricornutum* Front-End Desaturases Involved in Eicosapentaenoic Acid Biosynthesis", Eur. J. Biochem. 269 (2002), pp. 4105-4113.	
	CM**	Totani, N. et al., "The Filamentous Fungus *Mortierella alpina*, High in Arachiodonic Acid", Lipids, 22:2 (1987), pp. 1060-1062.	
	CN**	Cleland, L.G. et al., "Fish Oil and Rheumatoid Arthritis: Antiinflammatory and Collateral Health Benefits", The Journal of Rheumatology, 27:10 (2000), pp. 2305-2307.	
	CO**	Vazhappilly, R. et al., "Heterotrophic Production Potential of Omega-3 Polyunsaturated Fatty Acids by Microalgae and Algae-like Microorganisms", Botanica Marina 41 (1998), pp. 553-558.	
	CP**	Tvrdik, P. et al., "Role of a New Mammalian Gene Family in the Biosynthesis of Very Long Chain Fatty Acids and Sphingolipids", The Journal of Cell Biology, 149:3 (2000) pp. 707-717.	
	CQ**	Guehnemann-Schaefer, K. et al., "Fatty Acid β-oxidation in Glyoxysomes. Characterization of a New Tetrafunctional Protein (MFPIII)", Biochimica et Biophysica Acta 1256 (1995), pp. 181-186.	
	CR**	Meyer, A. et al., "Novel Fatty Acid Elongases and Their Use for the Reconstitution of Docosahexaenoic Acid Biosynthesis", Journal of Lipid Research 45 (2004), pp. 1899-1909.	

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First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	CS**	Sakuradani, E. et al., "Δ6-Fatty Acid Desaturase from an Arachidonic Acid-Producing *Mortierella* Fungus Gene Cloning and Its Heterologous Expression in a Fungus, *Aspergillus*", Gene 238 (1999), pp. 445-453.	
	CT**	Kinney, A.J., "Genetic Engeering of Oilseeds for Desired Traits", in "Genetic Engineering, Principles and Methods", Vol. 19, Editor: J. Setlow, (1997), pp. 149-166.	
	CU**	Voelker, T., "Plant Acyl-ACP Thioesterases: Chain-Length Determining Enzymes in Plant Fatty Acid Biosynthesis", in "Genetic Engineering, Principles and Methods", Vol. 18, Editor: J. Setlow, (1996), pp. 111-113.	
	CV**	Stukey, J.E. et al., "The *OLE1* Gene of *Saccharomyces cerevisiae* Encodes the Δ9 Fatty Acid Desaturase and Can Be Functionally Replaced by the Rat Stearoyl-CoA Desaturase Gene", The Jounal of Biological Chemistry 265:33 (1990), pp. 20144-20149.	
	CW**	Zank, T.K. et al., "Cloning and Functional Expression of the First Plant Fatty Acid Elongase Specific for Δ^6-Polyunsaturated Fatty Acids", Biochemical Society Transactions 28:6 (2000), pp. 654-658.	
	CX**	Poulos, A., "Very Long Chain Fatty Acids in Higher Animals - A Review", Lipids 30:1 (1995), pp. 1-14.	
	CY**	Huang, Y-S. et al., "Cloning of Δ12- and Δ6-Desaturases from *Mortierella alpina* and Recombinant Production of γ-Linolenic Acid in *Saccharomyces cerevisiae*", Lipids 34:7 (1999), pp. 649-659.	
	CZ**	Tocher, D.R. et al., "Recent Advances in the Biochemistry and Molecular Biology of Fatty Acyl Desaturases", Prog. Lipid Res. 37:2/3 (1998), pp. 73-117.	
	CA1**	Horrocks, L.A. et al., "Health Benefits of Docosahexaenoic Acid (DHA)", Pharmacological Research 40:3 (1999), pp. 211-225.	
	CB1**	McKeon, T. et al., "Stearoyl-Acyl Carrier Protein Desaturase from Safflower Seeds", in Methods in Enzymology, Vol. 71, Part C: Lipids, Editor: J. Lowenstein (1981), New York, pp. 275-281.	
	CC1**	Takeyama, H. et al., "Expression of the Eicosapentaenoic Acid Synthesis Gene Cluster from *Shewanella* sp. in a Transgenic Marine Cyanobacterium, *Synechococcus* sp.", Microbiology 143 (1997), pp. 2725-2731.	
	CD1**	Murphy, D.J. et al., "Biosynthesis, Targeting and Processing of Oleosin-like Proteins, Which are Major Pollen Coat Components in *Brassica napus*", Plant Journal 13:1 (1998), pp. 1-16.	
	CE1**	Wang, X.-M. et al., "Biosynthesis and Regulation of Linolenic Acid in Higher Plants", Physiol. Biochem. 26:6 (1988), pp. 777-792.	
	CF1**	Zank, T.K. et al., "Cloning and Functional Characterisation of an Enzyme Involved in the Elongation of Δ6-polyunsaturated Fatty Acids from the Moss *Physcomitrella patens*", The Plant Journal 31:3 (2002), pp. 255-268.	
	CG1**	Millar, A.A. et al., "*CUT1*, an Arabidopsis Gene Required for Cuticular Wax Biosynthesis and Pollen Fertility, Encodes a Very-Long-Chain Fatty Acid Condensing Enzyme", The Plant Cell 11 (1999), pp. 825-838.	
	CH1**	Calder, P.C., "Dietary Modification of Inflammation with Lipids", Proceedings of the Nutrition Society 61 (2002), pp. 345-358.	
	CI1**	Kunau, W.-H., et al., "β-oxidation of Fatty Acids in Mitochondria, Peroxisomes, and Bacteria: A Century of Continued Progress", Prog. Lipid Res. 34:4 (1995), pp. 267-342.	

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Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	CJ1**	Beaudoin, F. et al., "Heterologous Reconstitution in Yeast of the Polyunsaturated Fatty Acid Biosynthetic Pathway", Proceedings of the National Academy of Sciences of the United States of America 97:12 (2000), pp. 6421-6426.	
	CK1**	Ohlrogge, J. et al., "Lipid Biosynthesis", The Plant Cell 7 (1995), pp. 957-970.	
	CL1**	Millar, A.A. et al., "Very-long-chain Fatty Acid Biosynthesis is Controlled through the Expression and Specificity of the Condensing Enzyme", The Plant Journal 12:1 (1997), pp. 121-131.	
	CM1**	Shimokawa, H., "Beneficial Effects of Eicosapentaenoic Acid on Endothelial Vasodilator Functions in Animals and Humans", World Rev. Nutr. Diet 88 (2001), pp. 100-108.	
	CN1**	Chalova, L. I., et al. "The Composition of Lipids of Phytophthora infestans and Their Ability to Induce Potato Phytoalexin Accumulation". Biokhimiya, 1987, Vol. 52, No. 9, pp. 1445-1453; also see Database BIOSIS, Abstract No. PREV198885045135.	
	CO1**	Abbadi, A. et al., "Biosynthesis of Very-Long-Chain Polyunsaturated Fatty Acids in Transgenic Oilseeds: Constraints on Their Accumulation", The Plant Cell 16 (2004), pp. 2734-2748.	
	CP1**	"MY-26-A-10 PinfestansMY Phytophthora infestans cDNA, mRNA sequence." Database EMBL, Accession No. BE777235, September 21, 2000.	
	CQ1**	Domergue, F. et al., "Cloning and Functional Characterization of *Phaeodactylum tricornutum* Front-End Desaturases Involved in Eicosapentaenoic Acid Biosynthesis", Eur. J. Biochem. 269 (2002), pp. 4105-4113.	
	CR1**	Kamoun, S. et al., "Initial Assessment of Gene Diversity for the Oomycete Pathogen *Phytophthora infestans* Based on Expressed Sequences", Fungal Genetics and Biology 28 (1999), pp. 94-106.	
	CS1**	Khozin, I. et al., "Elucidation of the Biosynthesis of Eicosapentaenoic Acid in the Microalga *Porphyridium cruentum*", Plant Physiol. 114 (1997), pp. 223-230.	
	CT1**	Pereira, S.L. et al., "A Novel ω3-Fatty Acid Desaturase Involved in the Biosynthesis of Eicosapentaenoic Acid", Biochem. J. 378 (2004), pp. 665-671.	
	CU1**	Pereira, S.L. et al., "Recent Advances in the Study of Fatty Acid Desaturases from Animals and Lower Eukaryotes", Prostaglandins, Leukotrienes and Essential Fatty Acids 68 (2003), pp. 97-106.	
	CV1**	Spychalla, J.P. et al., "Identification of an Animal ω-3 Fatty Acid Desaturase by Heterologous Expression in *Arabidopsis*", Proc. Natl. Acad. Sci. USA 94 (1997), pp. 1142-1147.	
	CW1**	KAJIKAWA, M., et al., "Isolation and Functional Characterization of Fatty Acid Δ5-Elongase Gene from the Liverwort *Marchantia polymorpha* L.", FEBS Letters, 2006, Vol. 580, pp. 149-154.	
	CX1**	ROBERT, S. S., et al., "Isolation and Characterisation of a Δ5-Fatty Acid Elongase from the Marine Microalga *Pavlova salina*", Mar. Biotechnol., 2009, Vol. 11, pp. 410-418.	
	CY1**	PEREIRA, S. L., et al., "Identification of Two Novel Microalgal Enzymes Involved in the Conversion of the ω3-Fatty Acid, Eicosapentaenoic Acid, into Docosahexaenoic Acid", Biochem. J., 2004, Vol. 384, pp. 357-366.	
	CZ1**	LEONARD, A. E., et al., "Elongation of Long-Chain Fatty Acids", Progress in Lipid Research, 2004, Vol. 43, pp. 36-54.	
	CA2**	SPERLING, P., et al., "The Evolution of Desaturases", Prostaglandins, Leukotrienes and Essential Fatty Acids, 2003, Vol. 68, pp. 73-95.	
	CB2**	"LOC398440 Protein", UniProt Database Accession No. Q7ZXJ4, June 1, 2003.	

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Sheet 6 of 6

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Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	CC2**	DOMERGUE, F., et al., "New Insight into *Phaeodactylum tricornutum* Fatty Acid Metabolism. Cloning and Functional Characterization of Plastidial and Microsomal Δ12-Fatty Acid Desaturases", Plant Physiology, 2003, Vol. 131, pp. 1648-1660.	
	CD2**	WU, G., et al., "Stepwise Engineering to Produce High Yields of Very Long-Chain Polyunsaturated Fatty Acids in Plants", Nature Biotechnology, 2005, Vol. 23, pp. 1013-1017.	
	CE2**	NAKAMURA, M. T., et al., "Structure, Function, and Dietary Regulation of Δ6, Δ5, and Δ9 Desaturases", Annu. Rev. Nutr., 2004, Vol. 24, pp. 345-376.	
	CF2**	"P. patens Delta6 Elongase SEQ ID 29", GeneSeq Database Accession No. ABG73608, March 25, 2003.	
	CG2**	"Subname: Full = Polyunsaturated Fatty Acid Elongase elvol5a", UniProt Database Accession No. Q8AWE7, October 25, 2005.	
	CH2**	"Polyunsaturated Fatty Acid Elongase (ELOVL Family Member 5, Elongation of Long Chain Fatty Acids) (YEAST)", UniProt Database Accession No. Q8AX86, March 1, 2003.	
	CI2**	"633167 NCCCWA 1RT Oncorhynchus Mykiss cDNA Clone 1RT126D03_B_B02 5', mRNA Sequence", EMBL Database Accession No. CA360014, November 7, 2002.	
	CJ2**	HUANG, Y.-S., et al., "Enzymes for Transgenic Biosynthesis of Long-Chain Polyunsaturated Fatty Acids", Biochimie, 2004, Vol. 86, No. 11, pp. 793-798.	
	CK2**	"Physcomitrella patens Desaturase Encoding cDNA SEQ ID NO 7", GeneSeq Database Accession No. ABV74260, March 28, 2003.	
	CL2**	"Phaeodactylum tricornutum Desaturase Encoding cDNA SEQ ID NO 11", GeneSeq Database Accession No. ABV74262, March 28, 2003.	
	CM2**	Sprecher, H. "Metabolism of Highly Unsaturated n-3 and n-6 Fatty Acids", Biochimica et Biophysica Acta, 2000, Vol. 1486, pp. 219-231.	
	CN2**	"Nouveau Dictionnaire des Huiles Végétales: Compositions en Acides Gras", Ucciani E., Ed. Technique & Documentation - Lavoisier, 1995, ISBN: 2-7430-0009-0, pp. 577, 578 and 582.	
	CO2**	"Phaeodactylum tricornutum Elongase Encoding cDNA SEQ ID NO 9",GeneSeq Database Accession No. ABV74261, March 28, 2003.	
	CP2**	Bork, P., et al., "Go Hunting in Sequence Databases but Watch Out for the Traps", Trends in Genet. 12:10 (1996), pp. 425-427.	
	CQ2**	Broun, P., et al., "Catalytic Plasticity of Fatty Acid Modification Enzymes Underlying Chemical Diversity of Plant Lipids", Science 282:5392 (1998), pp. 1315-1317.	
	CR2**	van de Loo, F. J., et al., "An Oleate 12-Hydroxylase from *Ricinus communis* L. is a Fatty Acyl Desaturase Homolog", Proc. Natl. Acad. Sci. U S A 92:15 (1995), pp. 6743-6747.	
	CS2**	Doerks, T., et al., "Protein Annotation: Detective Work for Function Prediction", Trends in Genet. 14:6 (1998), pp. 248-250.	
	CT2**	Brenner, S. E., "Errors in Genome Annotation", Trends in Genet. 15:4 (1999), pp. 132-133.	
	CU2**	Yu, Z., et al., "Study on Nutritional Function of Polyunsaturated Fatty Acid", China Feed, 2003, Issue 24, pp. 21-23.	
	CV2**	Qi, B., et al., "Production of Very Long Chain Polyunsaturated Omega-3 and Omega-6 Fatty Acids in Plants", Nature Biotechnology, 2004, Vol. 22, No. 6, pp. 739-745.	
	CW2**	Robert, S. S., "Production of Eicosapentaenoic and Docosahexaenoic Acid-Containing Oils in Transgenic Land Plants for Human and Aquaculture Nutrition, Marine Biotechnology, 2006, 8: 103-109.	

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Method for producing polyunsaturated fatty acids

RELATED APPLICATIONS

This application is a continuation of patent application Serial No. 12/280,090 filed August 20, 2008, which is a national stage application (under 35 U.S.C. § 371) of PCT/EP2007/051675, filed February 21, 2007, which claims benefit of German application 10 2006 008 030.0, filed February 21, 2006 and European application 06120309.7, filed September 7, 2006. The entire content of each aforementioned application is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING

The Sequence Listing associated with this application is filed in electronic format *via* EFS-Web and hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is Sequence_Listing_074017_0013_01. The size of the text file is 730 KB, and the text file was created on September 2, 2016.

The present invention relates to a process for the production of eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in transgenic plants, providing in the plant at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity, where the nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified by comparison with the nucleic acid sequence in the organism from which the sequence is derived in that it is adapted to the codon usage in one or more plant species.

In a preferred embodiment there is additionally provision of further nucleic acid sequences which code for a polypeptide having the activity of an ω3-desaturase and/or of a Δ4-desaturase in the plant.

In a further preferred embodiment there is provision of further nucleic acid sequences which code for acyl-CoA dehydrogenase(s), acyl-ACP (acyl carrier protein) desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyl transferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A

carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) in the plant.

The invention furthermore relates to recombinant nucleic acid molecules comprising at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species.

A further part of the invention relates to oils, lipids and/or fatty acids which have been produced by the process according to the invention, and to their use.

Finally, the invention also relates to transgenic plants which have been produced by the process of the invention or which comprise a recombinant nucleic acid molecule of the invention, and to the use thereof as foodstuffs or feedstuffs.

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3-phosphate, and the addition or modification of a polar head group. Usual lipids which are used in membranes comprise phospholipids, glycolipids, sphingolipids and phosphoglycerides. Fatty acid synthesis starts with the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase or into acetyl-ACP by acetyl transacylase. After condensation reaction, these two product molecules together form acetoacetyl-ACP, which is converted via a series of condensation, reduction and dehydration reactions so that a saturated fatty acid molecule with the desired chain length is obtained. The production of the unsaturated fatty acids from these molecules is catalyzed by specific desaturases, either aerobically by means of molecular oxygen or anaerobically (regarding the fatty acid synthesis in microorganisms, see F.C. Neidhardt et al. (1996) E. coli and Salmonella. ASM Press: Washington, D.C., p. 612-636 and references cited therein; Lengeler et al. (Ed.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, and the references therein, and Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 and the references therein). To undergo the further elongation steps, the resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This is made possibly by acyl-CoA:lysophospho-

lipid acyltransferases. Moreover, these enzymes are capable of transferring the elongated fatty acids from the CoA esters back to the phospholipids. If appropriate, this reaction sequence can be followed repeatedly.

Furthermore, fatty acids must subsequently be transported to various modification sites and incorporated into the triacylglycerol storage lipid. A further important step during lipid synthesis is the transfer of fatty acids to the polar head groups, for example by glycerol fatty acid acyltransferase (see Frentzen, 1998, Lipid, 100(4-5):161-166).

An overview of the biosynthesis of fatty acids in plants, desaturation, the lipid metabolism and the membrane transport of lipidic compounds, beta-oxidation, the modification of fatty acids, cofactors and the storage and assembly of triacylglycerol, including the references is given by the following papers: Kinney (1997) Genetic Engineering, Ed.: JK Setlow, 19:149-166; Ohlrogge and Browse (1995) Plant Cell 7:957-970; Shanklin and Cahoon (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker (1996) Genetic Engeneering, Ed.: JK Setlow, 18:111-13; Gerhardt (1992) Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl (1995) Biochim. Biophys Acta 1256:181-186; Kunau et al. (1995) Prog. Lipid Res. 34:267-342; Stymne et al. (1993) in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Ed.: Murata und Somerville, Rockville, American Society of Plant Physiologists, 150-158; Murphy & Ross (1998) Plant Journal. 13(1):1-16.

Depending on the desaturation pattern, two large classes of polyunsaturated fatty acids, the ω6 and the ω3 fatty acids, which differ with regard to their metabolism and their function, can be distinguished.

In the text which follows, polyunsaturated fatty acids are referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (poly unsaturated fatty acids, PUFA, long chain poly unsaturated fatty acids, LCPUFA).

The fatty acid linoleic acid ($18:2^{\Delta 9,12}$) acts as starting material for the ω6 metabolic pathway, while the ω3 pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed from linoleic acid by the activity of an ω3-desaturase (Tocher et al. (1998) Prog. Lipid Res. 37: 73-117; Domergue et al. (2002) Eur. J. Biochem. 269: 4105-4113).

Mammals, and thus also humans, have no corresponding desaturase activity (Δ12- and ω3-desaturase) for the formation of the starting materials and must therefore take up these fatty acids (essential fatty acids) via the food. Starting with these precursors, the physiologically important polyunsaturated fatty acids arachidonic acid (= ARA, $20:4^{\Delta 5,8,11,14}$), an ω6-fatty

acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, $20:5^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA, $22:6^{\Delta 4,7,10,13,17,19}$) are synthesized via a sequence of desaturase and elongase reactions.

The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C_{20}- and C_{22}-PUFAs, respectively. This process proceeds via 4 steps. The first step is the condensation of malonyl-CoA onto the fatty acid acyl-CoA by ketoacyl-CoA synthase (KCS, hereinbelow referred to as elongase). This is followed by a reduction step (ketoacyl-CoA reductase, KCR), a dehydratation step (dehydratase) and a final reduction step (enoyl-CoA reductase). It has been postulated that the elongase activity affects the specificity and rate of the entire process (Millar and Kunst (1997) Plant Journal 12:121-131).

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and the pharmacological sector. Depending on whether they are free saturated or unsaturated fatty acids or else triacylglycerides with an elevated content of saturated or unsaturated fatty acids, they are suitable for very different applications. Thus, for example, lipids with unsaturated, specifically with polyunsaturated fatty acids, are preferred in human nutrition. The polyunsaturated ω3-fatty acids are supposed to have a positive effect on the cholesterol level in the blood and thus on the prevention of heart disease. The risk of heart disease, strokes or hypertension can be reduced markedly by adding these ω3-fatty acids to the food (Shimikawa (2001) World Rev. Nutr. Diet. 88: 100-108).

ω3-fatty acids also have a positive effect on inflammatory, specifically on chronically inflammatory, processes in association with immunological diseases such as rheumatoid arthritis (Calder (2002) Proc. Nutr. Soc. 61: 345-358; Cleland and James (2000) J. Rheumatol. 27: 2305-2307). They are therefore added to foodstuffs, specifically to dietetic foodstuffs, or are employed in medicaments. ω6-fatty acids such as arachidonic acid tend to have a negative effect in connection with these rheumatological diseases.

ω3- and ω6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG_2 series) which are formed from the ω6-fatty acids, generally promote inflammatory reactions, while eicosanoids (known as the PG_3 series) from ω3-fatty acids have little or no proinflammatory effect.

Polyunsaturated long-chain ω3-fatty acids such as eicosapentaenoic acid (= EPA, C20:$5^{\Delta5,8,11,14,17}$) or docosahexaenoic acid (= DHA, C22:$6^{\Delta4,7,10,13,16,19}$) are important components of human nutrition owing to their various roles in health aspects, including the development of the child brain, the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A (1995) Lipids 30:1-14; Horrocks, LA and Yeo YK (1999) Pharmacol Res 40:211-225).

Owing to the present-day composition of human food, an addition of polyunsaturated ω3-fatty acids, which are preferentially found in fish oils, to the food is particularly important. Thus, for example, polyunsaturated fatty acids such as docosahexaenoic acid (= DHA, C22:$6^{\Delta4,7,10,13,16,19}$) or eicosapentaenoic acid (= EPA, C20:$5^{\Delta5,8,11,14,17}$) are added to infant formula to improve the nutritional value. There is therefore a demand for the production of polyunsaturated long-chain fatty acids.

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella or Schizochytrium or from oil-producing plants such as soybeans, oilseed rape, and algae such as Crypthecodinium or Phaeodactylum and others, being obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, for example, fish. The free fatty acids are advantageously prepared by hydrolyzing the triacylglycerides. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (ARA, C20:$4^{\Delta5,8,11,14}$), dihomo-γ-linolenic acid (DHGL, C20:$3^{\Delta8,11,14}$) or docosapentaenoic acid (DPA, C22:$5^{\Delta7,10,13,16,19}$) are, however, not synthesized in oil crops such as oilseed rape, soybeans, sunflowers and safflower. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Owing to the positive characteristics of the polyunsaturated fatty acids, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of these fatty acids or triglycerides for the production of oils in various organisms with a modified content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describe a Δ9-desaturase. WO 93/11245 claims a Δ15-desaturase and WO 94/11516 a Δ12-desaturase. Further desaturates are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al. (1990) J. Biol. Chem., 265: 20144-20149, Wada et al. (1990) Nature 347: 200-203 or Huang et al. (1999) Lipids 34: 649-659. However, the biochemical characterization of the various desaturases has

been insufficient to date since the enzymes, being membrane-bound proteins, present great difficulty in their isolation and characterization (McKeon et al. (1981) Methods in Enzymol. 71: 12141-12147, Wang et al. (1988) Plant Physiol. Biochem., 26: 777-792).

As a rule, membrane-bound desaturases are characterized by being introduced into a suitable organism which is subsequently analyzed for enzyme activity by analyzing the starting materials and the products. Δ6-Desaturases are described in WO 93/06712, US 5,614,393, WO 96/21022, WO 00/21557 and WO 99/27111. The application of this enzyme for the production of fatty acids in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. The expression of various desaturases and the formation of polyunsaturated fatty acids is also described and claimed in WO 99/64616 or WO 98/46776. As regards the expression efficacy of desaturases and its effect on the formation of polyunsaturated fatty acids, it must be noted that the expression of a single desaturase as described to date has only resulted in low contents of unsaturated fatty acids/lipids such as, for example, γ-linolenic acid and stearidonic acid.

There have been a number of attempts in the past to obtain elongase genes. Millar and Kunst (1997) Plant Journal 12:121-131 and Millar et al. (1999) Plant Cell 11:825-838 describe the characterization of plant elongases for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the formation of waxes in plants (C_{28}-C_{32}). The synthesis of arachidonic acid and EPA is described, for example, in WO 01/59128, WO 00/12720, WO 02/077213 and WO 02/08401. The synthesis of polyunsaturated C24-fatty acids is described, for example, in Tvrdik et al. (2000) J. Cell Biol. 149:707-718 or in WO 02/44320.

Especially suitable microorganisms for the production of PUFAs are microalgae such as Phaeodactylum tricornutum, Porphiridium species, Thraustochytrium species, Schizochytrium species or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungi such as Mortierella, Entomophthora or Mucor and/or mosses such as Physcomitrella, Ceratodon and Marchantia (R. Vazhappilly & F. Chen (1998) Botanica Marina 41: 553-558; K. Totani & K. Oba (1987) Lipids 22: 1060-1062; M. Akimoto et al. (1998) Appl. Biochemistry and Biotechnology 73: 269-278). Strain selection has resulted in the development of a number of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFAs. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult process. Moreover, only limited

amounts of the desired polyunsaturated fatty acids such as DPA, EPA or ARA can be produced with the aid of the abovementioned microorganisms; in addition, they are generally obtained as fatty acid mixtures. This is why recombinant methods are preferred whenever possible.

Higher plants comprise polyunsaturated fatty acids such as linoleic acid (C18:2) and linolenic acid (C18:3). ARA, EPA and DHA are found not at all in the seed oil of higher plants, or only in miniscule amounts (E. Ucciani: Nouveau Dictionnaire des Huiles Végétales [New Dictionary of the Vegetable Oils]. Technique & Documentation – Lavoisier, 1995. ISBN: 2-7430-0009-0). However, the production of LCPUFAs in higher plants, preferably in oil crops such as oilseed rape, linseed, sunflowers and soybeans, would be advantageous since large amounts of high-quality LCPUFAs for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically. To this end, it is advantageous to introduce, into oilseeds, genes which encode enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. These genes encode for example Δ6-desaturases, Δ6-elongases, Δ5-desaturases or Δ4-desaturases. These genes can advantageously be isolated from microorganisms and lower plants which produce LCPUFAs and incorporate them in the membranes or triacylglycerides. Thus, it has already been possible to isolate Δ6-desaturase genes from the moss Physcomitrella patens and Δ6-elongase genes from P. patens and from the nematode C. elegans.

Transgenic plants which comprise and express genes encoding LCPUFA biosynthesis enzymes and which, as a consequence, produce LCPUFAs have been described, for example, in DE-A-102 19 203 (process for the production of polyunsaturated fatty acids in plants). However, these plants produce LCPUFAs in amounts which require further optimization for processing the oils which are present in the plants. Thus, the ARA content in the plants described in DE-A-102 19 203 is only 0.4 to 2% and the EPA content only 0.5 to 1%, in each case based on the total lipid content of the plant.

To make possible the fortification of food and of feed with polyunsaturated, long-chain fatty acids, there is therefore a great need for a simple, inexpensive process for the production of polyunsaturated, long-chain fatty acids, specifically in plant systems.

One object of the invention is therefore to provide a process with which long-chain polyunsaturated fatty acids, especially eicosapentaenoic acid, docosapentaenoic acid and/or

docosahexaenoic acid can be produced in large quantities and inexpensively in transgenic plants.

It has now surprisingly been found that the yield of long-chain polyunsaturated fatty acids, especially eicosapentaenoic, docosapentaenoic acid and/or docosahexaenoic acid, can be increased by expressing an optimized Δ5-elongase sequence in transgenic plants.

The PUFAs produced by the process of the invention comprise a group of molecules which higher animals are no longer able to synthesize and thus must consume, or which higher animals are no longer able to produce themselves in sufficient amounts and thus must consume additional amounts thereof, although they can easily be synthesized by other organisms such as bacteria.

Accordingly, the object of the invention is achieved by the process of the invention for producing eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in a transgenic plant, comprising the provision in the plant of at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity,

where the nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified by comparison with the nucleic acid sequence in the organism from which the sequence is derived in that it is adapted to the codon usage in one or more plant species. To produce DHA it is additionally necessary to provide at least one nucleic acid sequence which codes for a polypeptide having a Δ4-desaturase activity in the plant.

The "provision in the plant" means in the context of the present invention that measures are taken so that the nucleic acid sequences coding for a polypeptide having a Δ6-desaturase activity, a polypeptide having a Δ6-elongase activity, a polypeptide having a Δ5-desaturase activity and a polypeptide having a Δ5-elongase activity are present together in one plant. The "provision in the plant" thus comprises the introduction of the nucleic acid sequences into the plant both by transformation of a plant with one or more recombinant nucleic acid molecules which comprise said nucleic acid sequences, and by crossing suitable parent plants which comprise one or more of said nucleic acid sequences.

The nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified according to the invention by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species. This means that the nucleic acid sequence has been specifically optimized for the purpose of the invention without the amino acid sequence encoded by the nucleic acid sequence having been altered thereby.

The genetic code is redundant because it uses 61 codons in order to specify 20 amino acids. Therefore, most of the 20 proteinogenic amino acids are therefore encoded by a plurality of triplets (codons). The synonymous codons which specify an individual amino acid are, however, not used with the same frequency in a particular organism; on the contrary there are preferred codons which are frequently used, and codons which are used more rarely. These differences in codon usage are attributed to selective evolutionary pressures and especially the efficiency of translation. One reason for the lower translation efficiency of rarely occurring codons might be that the corresponding aminoacyl-tRNA pools are exhausted and thus no longer available for protein synthesis.

In addition, different organisms prefer different codons. For this reason, for example, the expression of a recombinant DNA derived from a mammalian cell frequently proceeds only suboptimally in E. coli cells. It is therefore possible in some cases to increase expression by replacing rarely used codons with frequently used codons. Without wishing to be bound to one theory, it is assumed that the codon-optimized DNA sequences make more efficient translation possible, and the mRNAs formed therefrom possibly have a greater half-life in the cell and therefore are available more frequently for translation. From what has been said above, it follows that codon optimization is necessary only if the organism in which the nucleic acid sequence is to be expressed differs from the organism from which the nucleic acid sequence is originally derived.

For many organisms of which the DNA sequence of a relatively large number of genes is known there are tables from which the frequency of use of particular codons in the respective organism can be taken. It is possible with the aid of these tables to translate protein sequences with relatively high accuracy back into a DNA sequence which comprises the codons preferred in the respective organism for the various amino acids of the protein. Tables on codon usage can be found inter alia at the following Internet address:

www.kazusa.or.ip/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelechon (Software Leto) or Geneart (Software GeneOptimizer).

Adaptation of the sequences to the codon usage in a particular organism can take place with the aid of various criteria. On the one hand, it is possible to use for a particular amino acid always the codon which occurs most frequently in the selected organism but, on the other hand, the natural frequency of the various codons can also be taken into account, so that all the codons for a particular amino acid are incorporated into the optimized sequence according to their natural frequency. Selection of the position at which a particular base triplet is used can take place at random in this case. The DNA sequence was adapted according to the invention taking account of the natural frequency of individual codons, it also being suitable to use the codons occurring most frequently in the selected organism.

It is particularly preferred for a nucleic acid sequence from *Ostreococcus tauri* which codes for a polypeptide having a Δ5-elongase activity, such as, for example, the polypeptide depicted in SEQ ID NO: 110, to be adapted at least to the codon usage in oilseed rape, soybean and/or flax. The nucleic acid sequence originally derived from *Ostreococcus tauri* is preferably the sequence depicted in SEQ ID NO: 109. The DNA sequence coding for the Δ5-elongase is adapted in at least 20% of the positions, preferably in at least 30% of the positions, particularly preferably in at least 40% of the positions and most preferably in at least 50% of the positions to the codon usage in oilseed rape, soybean and/or flax.

The nucleic acid sequence used is most preferably the sequence indicated in SEQ ID NO: 64.

It will be appreciated that the invention also encompasses those codon-optimized DNA sequences which code for a polypeptide having the activity of a Δ5-elongase and whose amino acid sequence is modified in one or more positions by comparison with the wild-type sequence but which still has substantially the same activity as the wild-type protein.

The nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41, preferably having the sequence depicted in SEQ ID NO: 1,b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 or 42, preferably in SEQ ID SEQ ID NO: 2,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated a) or b) above, in particular of the nucleic acid sequence indicated in SEQ ID NO: 1, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the sequence indicated in SEQ ID NO: 1, and

e) nucleic acid sequences which code for an amino acid sequence and which have at least one, for example 2, 3, 4, 5, 6, 7 or 8, preferably all of the amino acid pattern indicated in SEQ ID NO: 43, 44, 45, 46, 47, 48, 49 or 50.

Amino acid pattern means short amino acid sequences which preferably comprise less than 50, particularly preferably less than 40 and especially from 10 to 40 and even more preferably from 10 to 30 amino acids.

For the present invention, the identity is ascertained preferably over the full length of the nucleotide or amino acid sequences of the invention, for example for the nucleic acid sequence indicated in SEQ ID NO: 64 over the full length of 903 nucleotides.

The nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 171, 173, 175, 177, 179, 181 or 183, especially having the sequence depicted in SEQ ID NO: 171,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 172, 174, 176, 178, 180, 182 or 184, especially for the amino acid sequence indicated in SEQ ID NO: 172,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 1, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the

nucleic acid sequences indicated in a) or b) above, especially to the sequence indicated in SEQ ID NO: 171, and

e) nucleic acid sequences which code for an amino acid sequence and which have at least one, for example 2, 3, 4, 5, 6, 7 or 8, preferably all of the amino acid pattern indicated in SEQ ID NO: 185, 186, 187, 188, 189, 190, 191 or 192.

The nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity is in particular likewise a codon-optimized sequence according to the present invention, preferably the nucleic acid sequence depicted in SEQ ID NO: 122.

The nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 51, 53 or 55, preferably having the sequence depicted in SEQ ID NO: 51,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 52, 54 or 56, preferably for the amino acid sequence indicated in SEQ ID NO: 52,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated in a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 51, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the nucleic acid indicated under SEQ ID NO: 51, and

e) nucleic acid sequences which code for an amino acid sequence which have at least one, for example 2, 3, 4, 5, 6 or 7, preferably all of the amino acid pattern indicated in SEQ ID NO: 57, 58, 59, 60, 61, 62 or 63.

Further suitable nucleic acid sequences can be found by the skilled worker from the literature or the well-known gene libraries such as, for example, www.ncbi.nlm.nih.gov.

In a further preferred embodiment of the process, additionally one or more nucleic acid sequences which code for a polypeptide having the activity of an ω-3-desaturase and/or of a Δ4-desaturase are introduced into the plant.

The nucleic acid sequence which codes for a polypeptide having an ω-3-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 193 or 195, preferably the sequence depicted in SEQ ID NO: 193,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 194,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequence indicated in SEQ ID NO: 193 or 195 under stringent conditions, and

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95%, and especially at least 96%, 97%, 98% or 99%, identical to the sequence indicated in SEQ ID NO: 193 or 195.

The ω-3-desaturase advantageously used in the process of the invention makes it possible to shift from the ω-6 biosynthetic pathway to the ω-3 biosynthetic pathway, leading to a shift from $C_{18:2}$ to $C_{18:3}$ fatty acids. It is further advantageous for the ω-3-desaturase to convert a wide range of phospholipids such as phosphatidylcholine (= PC), phosphatidylinositol (= PIS) or phosphatidylethanolamine (= PE). Finally, desaturation products can also be found in the neutral lipids (= NL), that is to say in the triglycerides.

The nucleic acid sequence which codes for a polypeptide having a Δ4-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, preferably having the sequence depicted in SEQ ID NO: 77,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 78, 80, 82, 84, 86, 88, 90, 92 or 94, preferably for the amino acid sequence indicated in SEQ ID NO: 78,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated in a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 77, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least

91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the sequence indicated in SEQ ID NO: 77, and

e) nucleic acid sequences which code for an amino acid sequence which have at least one, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14, preferably all of the amino acid pattern indicated in SEQ ID NO: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

The Δ4-desaturase which is advantageously used in the process of the invention catalyzes the introduction of a double bond into the fatty acid docosapentaenoic acid, leading to formation of docosahexaenoic acid.

It is advantageous for the described process of the invention additionally to introduce further nucleic acids which code for enzymes of fatty acid or lipid metabolism into the plants in addition to the nucleic acid sequences which code for polypeptides having a Δ6-desaturase activity, a Δ6-elongase activity, a Δ5-desaturase activity and a Δ5-elongase activity, and to the nucleic acid sequences which are introduced if appropriate and which code for a polypeptide having an ω-3-desaturase activity and/or a Δ4-desaturase activity.

It is possible in principle to use all genes of fatty acid or lipid metabolism in combination with the nucleic acid sequences used in the process of the invention; genes of fatty acid or lipid metabolism selected from the group of acyl-CoA dehydrogenase(s), acyl-ACP (acyl carrier protein) desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s),acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) are preferably used in combination with the Δ6-elongase, Δ6-desaturase, Δ5-desaturase and the Δ5-elongase, and if appropriate the ω3-desaturase and/or the Δ4-desaturase, it being possible to use individual genes or a plurality of genes in combination.

The nucleic acids used in the process of the invention are advantageously expressed in vegetative tissues (somatic tissue). Vegetative tissue means in the context of this invention a tissue which is propagated through mitotic divisions. Tissue of this type also arises through asexual reproduction (apomixis) and propagation. Propagation is the term used when the number of individuals increases in consecutive generations. These individuals arising through asexual propagation are very substantially identical to their parents. Examples of such tissues are leaf, flower, root, stalk, runners above or below ground (side shoots, stolons), rhizomes,

buds, tubers such as root tubers or stem tubers, bulb, brood bodies, brood buds, bulbuls or turion. Such tissues may also arise through pseudo vivipary, true vivipary or vivipary caused by humans. However, seeds arising through agamospermy, as are typical of Asteraceae, Poaceae or Rosaceae, are also included among the vegetative tissues in which expression advantageously takes place. The nucleic acids used in the process of the invention are expressed to a small extent or not at all in generative tissue (germ line tissue). Examples of such tissues are tissues arising through sexual reproduction, i.e. meiotic cell divisions, such as, for example, seeds arising through sexual processes.

A small extent means that, compared with vegetative tissue, the expression measured at the RNA and/or protein level is less than 5%, advantageously less than 3%, particularly advantageously less than 2%, most preferably less than 1; 0.5; 0.25 or 0.125%.

The nucleic acid sequences are particularly preferably expressed in the leaves of the transgenic plants. This has the advantage that the LCPUFAs produced according to the invention can be taken in by animals and humans directly by consuming the leaves, and no previous processing of the plant material is necessary.

Expression of the nucleic acid sequences of the invention in the leaf can be achieved by using constitutive or leaf-specific promoters.

"Constitutive promoters" are promoters which make expression possible in a large number of, preferably in all, tissues over a substantial period during plant development, preferably throughout plant development. A promoter from a plant or from a plant virus is preferably used. The promoter of the CaMV (cauliflower mosaic virus) 35S transcript (Franck et al. (1980) Cell 21: 285-294), the 19S CaMV promoter (US 5,352,605), the actin promoter from rice (McElroy et al. (1990) Plant Cell 2: 163-171), the legumin B promoter (GenBank Acc. No. X03677), the agrobacterium nopaline synthase promoter, the TR dual promoter, the agrobacterium octopine synthase promoter, the ubiquitin promoter (Holtorf et al. (1995) Plant Mol. Biol. 29: 637-649), the Smas promoter, the cinnamoyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits, the pEMU promoter (Last et al. (1991) Theor. Appl. Genet. 81: 581-588), the MAS promoter (Velten et al. (1984) EMBO J. 3(12): 2723-2730), the histone H3 promoter from corn (Lepetit et al. (1992) Mol. Gen. Genet. 231: 276-285), the promoter of the nitrilase 1 gene from arabidopsis (GenBank Acc. No. U38846, nucleotides 3862-5325) and the promoter of a proline-rich protein from wheat

(WO 91/13991) and further promoters which mediate constitutive gene expression. The promoter of the CaMV 35S transcript is particularly preferred.

It is in principle possible to use all naturally occurring constitutive promoters with their regulatory sequences like those mentioned above for the novel process. However, it is likewise possible to use synthetic promoters in addition or alone.

"Leaf-specific promoters" are promoters which show a high activity in the leaf and no or only low activity in other tissues. "Low activity" means in the context of the invention that the activity in other tissues is less than 20%, preferably less than 10%, particularly preferably less than 5% and most preferably less than 3, 2 or 1% of the activity in the leaf. Examples of suitable leaf-specific promoters are the promoters of the small subunit of rubisco (Timko et al. (1985) Nature 318: 579-582) and of the chlorophyll a/b-binding protein (Simpson et al. (1985) EMBO J. 4: 2723-2729).

The skilled worker is aware of further leaf-specific promoters, or he can isolate further suitable promoters with known methods. Thus, the skilled worker is able to identify leaf-specific regulatory nucleic acid elements with the aid of conventional methods of molecular biology, e.g. hybridization experiments or DNA-protein binding studies. This entails for example in a first step isolating the total poly(A)$^+$ RNA from leaf tissue of the desired organism from which the regulatory sequences are to be isolated, and setting up a cDNA library. In a second step, cDNA clones which are based on poly(A)$^+$ RNA molecules from a non-leaf tissue are used to identify, by means of hybridization, those clones from the first library whose corresponding poly(A)$^+$ RNA molecules accumulate only in leaf tissue. Subsequently, these cDNAs identified in this way are used to isolate promoters which have leaf-specific regulatory elements. Further PCR-based methods for isolating suitable leaf-specific promoters are additionally available to the skilled worker.

It is, of course, also possible for the nucleic acid sequences of the present invention to be expressed in the seeds of the transgenic plants by using seed-specific promoters which are active in the embryo and/or in the endosperm. Seed-specific promoters can in principle be isolated both from dicotyledonous and from monocotyledonous plants. Preferred promoters are listed hereinafter: USP (unknown seed protein) and vicilin (Vicia faba) (Bäumlein et al. (1991) Mol. Gen Genet. 225(3): 459-467), napin (oilseed rape) (US 5,608,152), conlinin (flax) (WO 02/102970), acyl-carrier protein (oilseed rape) (US 5,315,001 and WO 92/18634), oleosin (Arabidopsis thaliana) (WO 98/45461 and WO 93/20216), phaseolin (Phaseolus

vulgaris) (US 5,504,200), Bce4 (WO 91/13980), legume B4 (LegB4 promoter) (Bäumlein et al. (1992) Plant J. 2(2): 233-239), Lpt2 and lpt1 (barley) (WO 95/15389 and WO 95/23230), seed-specific promoters from rice, corn and wheat (WO 99/16890), Amy32b, Amy 6-6 and aleurain (US 5,677,474), Bce4 (oilseed rape) (US 5,530,149), glycinin (soybean) (EP 571 741), phosphoenolpyruvate carboxylase (soybean) (JP 06/62870), ADR 12-2 (soybean) (WO 98/08962), isocitrate lyase (oilseed rape) (US 5,689,040) or α-amylase (barley) (EP 781 849).

In a particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of produced LCPUFAs, especially of the C22 fatty acids, in the seed oil is at least 5% by weight, advantageously at least 6, 7, 8, 9 or 10% by weight, preferably at least 11, 12, 13, 14 or 15% by weight, particularly preferably at least 16, 17, 18, 19 or 20% by weight, very particularly preferably at least 25, 30, 35 or 40% by weight, of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of C22 fatty acids in the seed oil is at least 8% by weight of the seed oil content.

In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of docosahexaenoic acid in the seed oil is at least 1% by weight, preferably at least 1.1, 1.2, 1.3, 1.4 or 1.5% by weight, particularly preferably at least 1.6, 1.7, 1.8 or 1.9% by weight, especially at least 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8 or 2.9% by weight, further preferably at least 3, 3.5 or 4% by weight of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of docosahexaenoic acid in the seed

oil is at least 1.9% by weight of the seed oil content. It is known to the skilled worker in this connection that to produce docosahexaenoic acid additionally one or more nucleic acid sequences which codes for a polypeptide having the activity of a Δ4-desaturase activity are required. A nucleic acid sequence which codes for a polypeptide having the activity of a Δ4-desaturase activity is advantageously selected from the group consisting of nucleic acid sequences having the sequence depicted in SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, preferably having the sequence depicted in SEQ ID NO: 77.

In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of docosahexaenoic acid in the seed oil is at least 1% by weight, preferably at least 1.1, 1.2, 1.3, 1.4 or 1.5% by weight, particularly preferably at least 1.6, 1.7, 1.8 or 1.9% by weight, especially at least 2, 2.1, 2.2, 2.5, 2.6, 2.7, 2.8 or 2.9% by weight, further preferably at least 3, 3.5 or 4% by weight of the seed oil content. In this case, the content of the produced LCPUFAs, especially of the C22 fatty acids, in the seed oil is at least 5% by weight, advantageously at least 6, 7, 8, 9 or 10% by weight, preferably at least 11, 12, 13, 14 or 15% by weight, particularly preferably at least 16, 17, 18, 19 or 20% by weight, very particularly preferably at least 25, 30, 35 or 40% by weight of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of docosahexaenoic acid in the seed oil is at least 1.9% by weight of the seed oil content, with the content of C22 fatty acids in the seed oil being at least 8% by weight of the seed oil content.

Plant gene expression can also be achieved via a chemically inducible promoter (see a review in Gatz (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that the gene expression takes place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

Promoters which respond to biotic or abiotic stress conditions are also suitable, for example the pathogen-induced PRP1 gene promoter (Ward et al., Plant. Mol. Biol. 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the chill-inducible potato alpha-amylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Other promoters which are also particularly suitable are those which bring about the plastid-specific expression, since plastids constitute the compartment in which precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters, such as the viral RNA polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the Arabidopsis clpP promoter, described in WO 99/46394.

It will be appreciated that the polyunsaturated fatty acids produced according to the invention can be produced not only in intact transgenic plants but also in plant cell cultures or in callous cultures.

The polyunsaturated fatty acids produced in the process are advantageously bound in phospholipids and/or triacylglycerides, but may also occur as free fatty acids or else bound in the form of other fatty acid esters in the organisms. They may in this connection be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different phospholipids such as phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and/or phosphatidylserine and/or triacylglycerides, monoacyl-glycerides and/or diacylglycerides. The LCPUFAs EPA, DPA and DHA produced in the process are advantageously present in phosphatidylcholine and/or phosphatidylethanolamine and/or in the triacylglycerides. The triacylglycerides may additionally also comprise further fatty acids such as short-chain fatty acids having 4 to 6 C atoms, medium-chain fatty acids having 8 to 12 C atoms or long-chain fatty acids having 14 to 24 C atoms. They preferably comprise long-chain fatty acids, particularly preferably C_{20} or C_{22} fatty acids.

The term "glyceride" is understood as meaning glycerol esterified with one, two or three carboxyl radicals (mono-, di- or triglyceride). "Glyceride" is also understood as meaning a mixture of various glycerides. The glyceride is preferably a triglyceride. The glyceride or glyceride mixture can comprise further additions, for example free fatty acids, antioxidants, proteins, carbohydrates, vitamins and/or other substances.

A"glyceride" for the purposes of the process according to the invention is furthermore understood as meaning derivatives which are derived from glycerol. In addition to the above-

described fatty acid glycerides, these also include glycerophospholipids and glyceroglycolipids. Preferred examples which may be mentioned here are the glycerophospholipids such as lecithin (phosphatidylcholine), cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylacylglycerophospholipids.

Phospholipids are to be understood as meaning, for the purposes of the invention, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and/or phosphatidylinositol.

The fatty acid esters with polyunsaturated C_{18}-, C_{20}- and/or C_{22}-fatty acid molecules can be isolated in the form of an oil or lipid, for example in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three or four, preferably four, five or six double bonds, from the useful plants which have been used for the preparation of the fatty acid esters; advantageously, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of the phosphatidyl ester, especially preferably in the form of the triacylglycerides, phosphatidylcholine and/or phosphatidylethanolamine. In addition to these esters, the polyunsaturated fatty acids are also present in the plants as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

The LCPUFAs produced in the process of the invention are produced with a content of at least 4% by weight, advantageously of at least 5, 6, 7, 8, 9 or 10% by weight, preferably of at least 11, 12, 13, 14 or 15% by weight, particularly preferably of at least 16, 17, 18, 19, or 20% by weight, very particularly preferably of at least 25, 30, 35 or 40% by weight based on the total fatty acids in the transgenic plant. The fatty acids EPA, DPA and/or DHA produced in the process of the invention are moreover present with a content of in each case at least 5% by weight, preferably of in each case at least 6, 7, 8 or 9% by weight, particularly preferably

of in each case at least 10, 11 or 12% by weight, most preferably of in each case at least 13, 14, 15, 16, 17, 18, 19 or 20% by weight based on the total fatty acids in the transgenic plant.

The fatty acids are advantageously produced in bound form. It is possible with the aid of the nucleic acids used in the process of the invention for these unsaturated fatty acids to be put on the sn1, sn2 and/or sn3 position of the advantageously produced triacylglycerides. Advantageously, at least 11% of the triacylglycerides are doubly substituted (meaning on the sn1 and sn2 or sn2 and sn3 positions). Triply substituted triacylglycerides are also detectable. Since a plurality of reaction steps take place from the starting compounds linoleic acid (C18:2) and linolenic acid (C18:3), the final products of the process, such as, for example, arachidonic acid (ARA) or eicosapentaenoic acid (EPA), do not result as absolute pure products; traces or larger amounts of the precursors are always also present in the final product. If, for example, both linoleic acid and linolenic acid are present in the initial plant, the final products such as ARA or EPA and/or DPA and/or DHA are also present as mixtures. The precursors should advantageously amount to not more than 20% by weight, preferably not more than 15% by weight, particularly preferably not as 10% by weight, very particularly preferably not more than 5% by weight based on the amount of the respective final product. Advantageously, only ARA or EPA and/or DPA and/or DHA are produced in the process of the invention, bound or as free acids, as final products in a transgenic plant.

Fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise 6 to 15% palmitic acid, 1 to 6% stearic acid; 7-85% oleic acid; 0.5 to 8% vaccenic acid, 0.1 to 1% arachic acid, 7 to 25% saturated fatty acids, 8 to 85% monounsaturated fatty acids and 60 to 85% polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Preferably at least 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 or 1% arachidonic acid in the total fatty acid content, are present as advantageous polyunsaturated fatty acid in the fatty acid ester or fatty acid mixtures. The fatty acid esters or fatty acid mixtures produced by the process of the invention further advantageously comprise fatty acids selected from the group of fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enonic acid), malvalic acid (8,9-methyleneheptadec-8-enonic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienonic acid), vernonic acid (9,10-epoxyoctadec-12-enonic acid), taric acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynonic acid, pyrulic acid (t10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-

13-ene-9,11-diynonic acid, petroselenic acid (cis-6-octadecenonic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid, catalpic acid (9t11t13c-octadecatrienoic acid), eleosteric acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienonic acid). In general, the aforementioned fatty acids are advantageously present only in traces in the fatty acid esters or fatty acid mixtures produced by the process of the invention, meaning that their occurrence, based on the total fatty acid content, is less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, particularly preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very particularly preferably less than 4%, 3%, 2% or 1%. In a further preferred form of the invention the occurrence of these aforementioned fatty acids, based on the total fatty acids, is less than 0.9%; 0.8%; 0.7%; 0.6% or 0.5%, particularly preferably less than 0.4%; 0.3%; 0.2%; 0.1%. The fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise less than 0.1% based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, C23:6$^{\Delta 3,8,12,15,18,21}$).

It is possible through the nucleic acid sequences used in the process of the invention to achieve an increase in the yield of LCPUFAs in the transgenic plants of at least 50%, advantageously of at least 80%, particularly advantageously of at least 100%, very particularly advantageously of at least 150%, compared with the non-transgenic plants.

Chemically pure polyunsaturated fatty acids or fatty acid compositions can also be synthesized by the processes described above. To this end, the fatty acids or the fatty acid compositions are isolated from the plants in the known manner, for example via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

In principle, all dicotyledonous or monocotyledonous useful plants are suitable for the process of the invention. Useful plants mean plants which serve to produce foods for humans and animals, to produce other consumables, fibers and pharmaceuticals, such as cereals, e.g. corn, rice, wheat, barley, millet, oats, rye, buckwheat; such as tubers, e.g. potato, cassava, sweet potato, yams etc.; such as sugar plants e.g. sugarcane or sugarbeet; such as legumes,

e.g. beans, peas, broad bean etc.: such as oil and fat crops, e.g. soybean, oilseed rape, sunflower, safflower, flax, camolina etc., to mention only a few. Advantageous plants are selected from the group of plant families consisting of the families of Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreacea, Elaeagnaceae, Ericceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae and Valerianaceae.

Examples which may be mentioned are the following plants: Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica (mango) or Anacardium occidentale (cashew), Asteraceae such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, e.g. the genus and species Calendula officinalis (common marigold), Carthamus tinctorius (safflower), Centaurea cyanus (cornflower), Cichorium intybus (chicory), Cynara scolymus (artichoke), Helianthus annus (sunflower), Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactus scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta (lettuce), Tagetes lucida, Tagetes erecta or Tagetes tenuifolia (French marigold), Apiaceae such as the genus Daucus, e.g. the genus and species Daucus carota (carrot), Betulaceae such as the genus Corylus, e.g. the genera and species Corylus avellana or Corylus colurna (hazelnut), Boraginaceae such as the genus Borago, e.g. the genus and species Borago officinalis (borage), Brassicaceae such as the genera Brassica, Camelina, Melanosinapis, Sinapis, Arabadopsis, e.g. the genera and species Brassica napus, Brassica rapa ssp. (oilseed rape), Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Camelina sativa, Melanosinapis communis (mustard), Brassica oleracea (feed beet) or Arabidopsis thaliana, Bromeliaceae such as the genera Anana, Bromelia (pineapple), e.g. the genera and species Anana comosus, Ananas ananas or Bromelia comosa (pineapple), Caricaceae such as the genus Carica such as the genus and species Carica papaya (papaya), Cannabaceae such as the genus Cannabis such as the genus and species Cannabis sative (hemp), Convolvulaceae such as the genera Ipomoea, Convolvulus, e.g. the genera and

species Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus (sweet potato, batate), Chenopodiaceae such as the genus Beta such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var. vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta (sugarbeet), Cucurbitaceae such as the genus Cucubita, e.g. the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata (pumpkin), Elaeagnaceae such as the genus Elaeagnus, e.g. the genus and species Olea europaea (olive), Ericaceae such as the genus Kalmia, e.g. the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros or Kalmia lucida (mountain laurel), Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, e.g. the genera and species Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta (cassava) or Ricinis communis (castor oil plant), Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, e.g. the genera and species Pisum sativum, Pisum arvense, Pisum humile (pea), Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa (acacia), Medicago sativa, Medicago falcata, Medicago varia (alfalfa) Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max (soybean), Geraniaceae such as the genera Pelargonium, Cocos, Oleum, e.g. the genera and species Cocos nucifera, Pelargonium grossularioides or Oleum cocois (coconut), Gramineae such as the genus Saccharum, e.g. the genus and species Saccharum officinarum, Juglandaceae such as the genera Juglans, Wallia, e.g. the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra or Wallia nigra (walnut), Lauraceae such as the genera Persea, Laurus, e.g. the genera and species Laurus nobilis (bay), Persea americana, Persea gratissima or Persea persea (avocado), Leguminosae such as the genus Arachis. e.g. the genus and species Arachis hypogaea (peanut), Linaceae such as the genera Linum, Adenolinum,

e.g. the genera and species Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or Linum trigynum (flax), Lythrarieae such as the genus Punica, e.g. the genus and species Punica granatum (pomegranate), Malvaceae such as the genus Gossypium, e.g. the genera and species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi (cotton), Musaceae such as the genus Musa, e.g. the genera and species Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. (banana), Onagraceae such as the genera Camissonia, Oenothera, e.g. the genera and species Oenothera biennis or Camissonia brevipes (evening primrose), Palmae such as the genus Elaeis, e.g. the genus and species Elaeis guineensis (oil palm), Papaveraceae such as the genus Papaver, e.g. the genera and species Papaver orientale, Papaver rhoeas, Papaver dubium (poppy), Pedaliaceae such as the genus Sesamum e.g the genus and species Sesamum indicum (sesame), Piperaceae such as the genera Piper, Artanthe, Peperomia, Steffensia, e.g. the genera and species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata (cayenne pepper), Poaceae such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (corn), triticum, e.g. the genera and species Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum (barley), Secale cereale (rye), Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida (oats), Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum (millet), Oryza sativa, Oryza latifolia (rice), Zea mays (corn), Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare (wheat), Porphyridiaceae such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, e.g. the genus and species Porphyridium cruentum, Proteaceae such as the genus Macadamia, e.g. the genus and species Macadamia

intergrifolia (macadamia), Rubiaceae such as the genus Coffea, e.g. the genera and species Coffea spp., Coffea arabica, Coffea canephora or Coffea liberica (coffee), Scrophulariaceae such as the genus Verbascum, e.g. the genera and species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus (mullein), Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, e.g. the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens (pepper), Capsicum annuum (paprika), Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris (tobacco), Solanum tuberosum (potato), Solanum melongena (aubergine), Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solanum lycopersicum (tomato), Sterculiaceae such as the genus Theobroma, e.g. the genus and species Theobroma cacao (cocoa), or Theaceae such as the genus Camellia, e.g. the genus and species Camellia sinensis (tea).

In an advantageous embodiment of the process, the useful plants used are oil fruit plants which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower (Carthamus tinctoria), poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, verbascum, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, flax, soybean, pistachios, borage, trees (oil palm, coconut or walnut) or arable crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, egg plant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Advantageous plants according to the invention are oil fruit plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, flax, soybean, borage, trees (oilpalm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as sunflower, safflower, tobacco, verbascum, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, flax, hemp or thistle. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, flax, or hemp.

It is also advantageous to express the nucleic acid sequences of the invention in the leaves of feed or food plants and thus to increase the content of eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in the leaves. Preferred feed plants are, for example, trefoil species such as red clover (Trifolium pratense), white clover (Trifolium repens), alsike clover (Trifolium hybridum), sainfoin (Onobrychis viccifolia), Egyptian clover (Trifolium alexandrinium) and Persian clover (Trifolium resupinatum). Preferred food plants are for instance lettuce species such as Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis and Valeriana locusta.

It is possible through the enzymatic activity of the nucleic acid sequences which are used in the process of the invention and which code for polypeptides having Δ6-elongase, Δ6-desaturase, Δ5-desaturase and/or Δ5-elongase activity, advantageously in combination with nucleic acid sequences which code for polypeptides having ω3-desaturase and/or Δ4-desaturase activity, and further nucleic acid sequences which code for polypeptides of fatty acid or lipid metabolism, such as further polypeptides having Δ5-, Δ6-, Δ8-, Δ12-desaturase or Δ5-, Δ6- or Δ9-elongase activity, to produce a wide variety of polyunsaturated fatty acids in the process of the invention. Depending on the useful plants chosen for use in the process of the invention, mixtures of the various polyunsaturated fatty acids or individual polyunsaturated fatty acids such as EPA, DPA or DHA can be produced in free or bound form. Depending on the prevailing fatty acid composition in the starting plant (C18:2 or C18:3 fatty acids), the resulting fatty acids are derived from C18:2 fatty acids, such as GLA, DGLA or ARA or are derived from C18:3 fatty acids, such as EPA, DPA or DHA. If the only unsaturated fatty acid present in the plant used for the process is linoleic acid (LA, $C18:2^{\Delta 9,12}$), the only possible products of the process are GLA, DGLA and ARA, which may be present as free fatty acids or bound. If the only unsaturated fatty acid present in the plant used in the process is α-linolenic acid (ALA, $C18:3^{\Delta 9,12,15}$), for example as in flax, the only possible products of the process are SDA, ETA, EPA, DPA and/or DHA, which may be present as described above as free fatty acids or bound. It is possible to produce in a targeted manner only individual products in the plant by modifying the activity of the enzymes used in the process and involved in the synthesis Δ6-elongase, Δ6-desaturase, Δ5-desaturase and/or Δ6-elongase, advantageously in combination with further genes of lipid or fatty acid metabolism. Advantageously, only EPA, DPA or DHA or mixtures thereof are synthesized. Since the fatty acids are synthesized in biosynthesis chains, the respective final products are

not present as pure substances in the organisms. Small amounts of the precursor compounds are always also present in the final product. These small amounts are less than 20% by weight, advantageously less than 15% by weight, particularly advantageously less than 10% by weight, very particularly advantageously less than 5, 4, 3, 2 or 1% by weight based on the final products EPA, DPA or DHA or mixtures thereof.

To increase the yield in the process according to the invention for the production of oils and/or triglycerides with a polyunsaturated fatty acid, content which is advantageously increased, it is advantageous to increase the amount of starting product for the synthesis of fatty acids. This can be achieved for example by introducing a nucleic acid which encodes a polypeptide with Δ12-desaturase into the organism. This is particularly advantageous in useful plants, such as oil-producing plants such as plants of the Brassicaceae family, such as the genus Brassica, for example rape; the Elaeagnaceae family, such as the genus Elaeagnus, for example the genus and species *Olea europaea* or the family Fabaceae, such as the genus Glycine, for example the genus and species *Glycine max*, which are high in oleic acid. Since these organisms have an only low linoleic acid content (Mikoklajczak et al. (1961) Journal of the American Oil Chemical Society 38: 678 - 681) it is advantageous to use said Δ12-desaturases for producing the starting material linolenic acid from oleic acid. It is also possible in addition for the starting fatty acids to be provided from outside, but this is less preferred for reasons of cost.

Mosses and algae are the only plant systems known to produce considerable amounts of polyunsaturated fatty acids such as arachidonic acid (ARA) and/or eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Mosses comprise PUFAs in membrane lipids, whereas algae, organisms related to algae, and some fungi also accumulate substantial amounts of PUFAs in the triacylglycerol fraction. Nucleic acid molecules isolated from strains which accumulate PUFAs also in the triacylglycerol fraction are therefore particularly advantageous for the process of the invention and thus for modifying the lipid and PUFA production system in a plant such as a useful plant such as an oil crop plant, for example oilseed rape, canola, flax, hemp, soybean, sunflower, borage. They can therefore advantageously be used in the process of the invention.

Nucleic acids used in the process of the invention are advantageously derived from plants such as algae, for example algae of the family of Prasinophyceae such as from the genera Heteromastix, Mammella, Mantoniella, Micromonas, Nephroselmis, Ostreococcus, Prasinocladus, Prasinococcus, Pseudoscourfielda, Pycnoocus, Pyramimonas, Scherffelia or

Tetraselmis such as the genera and species Heteromastix longifillis, Mamiella gilva, Mantoiella squamata, Micromonas pusilla, Nephroselmis olivacea, Nephroselmis pyriformis, Neproselmis rotunda, Ostreococcus tauri, Ostreococcus sp. Prasinocladus ascus, Prasinocladus lubricus, Pycnococcus provasolii, Pyramimonas amylifera, Pyramimonas disomata, Pyramimonas obovata, Pyramimonas orientalis, Pyramimonas parkae, Pyramimonas spinefera, Pyramimonas sp., Tetraselmis apiculta, Tetraselmis carteriaformis, Tetraselmis chui, Tetraselmis convolutae, Tetraselmis desikacharyi, Tetraselmis gracilis, Tetraselmis hazeni, Tetraselmis impellucida, Tetraselmis inconspicua, Tetraselmis levis, Tetraselmis maculata, Tetraselmis marina, Tetraselmis striata, Tetraselmis subcordiformis, Tetraselmis suecica, Tetraselmis tetrabrachia, Tetraselmis tetrathele, Tetraselmis verrucosa, Tetraselmis verrucosa fo. rubens or Tetraselmis sp. or algae from the family Euglenacease such as from the genera Ascoglena, Astasia, Colacium, Cyclidiopsis, Euglena, Euglenopsis, Hyalophacus, Khawkinea, Lepocinclis, Phacus, Strombomonas or Trachelomonas such as the genera and species Euglena acus, Euglena geniculata, Euglena gracilis, Euglena mixocylindracea, Euglena rostrifera, Euglena viridis, Colacium stentorium, Trachelomonas cylindrica or Trachelomonas volvocina.

Further advantageous plants are algae such as Isochrysis or Crypthecodinium, algae/diatoms such as Thalassiosira or Phaeodactylum, mosses such as Physcomitrella or Ceratodon or higher plants such as the Primulaceae such as Aleuritia, Calendula stella, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi such as Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor or Mortierella, bacteria such as shewanella, yeasts or animals such as nematodes such as Caenorhabditis, insects, frogs, sea cucumbers or fishes. The nucleic acid sequences isolated according to the invention are advantageously derived from an animal from the order of vertebrates. The nucleic acid sequences are preferably derived from the class of Vertebrata; Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus or Vertebrata, Amphibia, Anura, Pipidae, Xenopus or Evertebrata such as Protochordata, Tunicata, Holothuroidea, Cionidae such as Amaroucium constellatum, Botryllus schlosseri, Ciona intestinalis, Molgula citrina, Molgula manhattensis, Perophora viridis or Styela partita. The nucleic acids are particularly advantageously derived from fungi, animals or from plants such as algae or mosses, preferably from the order of Salmoniformes such as of the family of Salmonidae such as of the genus Salmo, for example from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario, from algae such as the

genera Mantoniella or Ostreococcus or from the diatoms such as the genera Thalassiosira or Phaeodactylum or from algae such as Crypthecodinium.

In a preferred embodiment, the process further comprises the step of obtaining a cell or a whole plant which comprises the nucleic acid sequences which are used in the process and which code for a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase and, if appropriate, nucleic acid sequences which code for an ω3-desaturase and/or a Δ4-desaturase, it being possible for the cell and/or the useful plant also to comprise further nucleic acid sequences of lipid or fatty acid metabolism. The nucleic acid sequences preferably used in the process are for expression advantageously incorporated into at least one gene construct and/or a vector as described hereinafter, alone or in combination with further nucleic acid sequences which code for proteins of fatty acid or lipid metabolism, and finally transformed into the cell or plant. In a further preferred embodiment, this process further comprises the step of obtaining the oils, lipids or free fatty acids from the useful plants, The cell produced in this way or the useful plant produced in this way is advantageously a cell of an oil-producing plant, vegetable plant, lettuce plant, or ornamental plant or the plant itself as stated above.

Growing means for the cultivation in the case of plant cells, tissue or organs on or in a nutrient medium or of the whole plant on or in a substrate, for example in hydroculture, flower pot soil or on an arable field.

For the purposes of the invention, "transgenic" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (= gene construct) or a vector comprising the nucleic acid sequences used in the process according to the invention or a plant transformed with the nucleic acid sequences, expression cassette or vector used in the process according to the invention, all those constructions brought about by recombinant methods in which either

a) the nucleic acid sequence, or

b) a genetic control sequence which is operably linked with the nucleic acid sequence, for example a promoter, or

c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to be, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic

environment means the natural genomic or chromosomal locus in the original organism or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, very especially preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequence used in the process according to the invention with the nucleic acid sequence which encodes proteins with corresponding Δ6-desaturase, Δ6-elongase, Δ5-desaturase and Δ5-elongase activity, advantageously in combination with nucleic acid sequences which encode proteins having ω3-desaturase and/or Δ4-desaturase activity – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

A "transgenic plant" for the purposes of the invention is understood as mentined above as meaning that the nucleic acids used in the process are not at their natural locus in the genome of the plant. In this case, it is possible for the nucleic acid sequences to be expressed homologously or heterologously. However, transgenic also means that, while the nucleic acids according to the invention are at their natural position in the genome of the plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids used in the process according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acid sequences takes place.

Preferred transgenic organisms are useful plants such as oil-producing plants, vegetable plants, lettuce plants or ornamental plants which are advantageously selected from the group of plant families consisting of the families of Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae,

Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae and Valerianaceae.

Host plants which are suitable for the nucleic acids, the expression cassette or the vector used in the process according to the invention are, in principle, advantageously all useful plants which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and which are suitable for the expression of recombinant genes. Examples which should be mentioned at this point are plants such as Arabidopsis, Asteraceae such as Calendula or useful plants such as soybean, peanut, castor-oil plant, sunflower, maize, cotton, flax, oilseed rape, coconut, oil palm, safflower (Carthamus tinctorius) or cacao bean. Further advantageous plants are mentioned at other points in this application.

Microorganisms are generally used as intermediate hosts for the production of transgenic useful plants. Such utilizable intermediate host cells are detailed in: Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990).

Expression strains which can advantageously be used for this purpose are, for example, those with a lower protease activity. They are described, for example, in: Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128.

Transgenic plants which comprise the polyunsaturated, long-chain fatty acids synthesized in the process according to the invention can advantageously be marketed directly without there being any need for the oils, lipids or fatty acids synthesized to be isolated. This form of marketing is particularly advantageous.

"Plants" for the purposes of the present invention are intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue.

The compounds produced in the process of the invention can, however, also be isolated from the plants in the form of their oils, fat, lipids and/or free fatty acids. Polyunsaturated fatty acids produced by the process of the invention can be obtained by harvesting the plants or plant cells either from the culture in which they grow or from the field. This can take place by

pressing or extracting the plant parts, preferably the plant seeds. It is possible in this connection for the oils, fats, lipids and/or free fatty acids to be obtained by pressing by so-called cold drawing or cold pressing without input of heat. To make it easier to break open the plant parts, specifically the seeds, they are previously crushed, steamed or roasted. The seeds pretreated in this way can then be pressed or extracted with solvent such as warm hexane. The solvent is then removed again. It is possible in this way to isolate more than 96% of the compounds produced in the process of the invention. The products obtained in this way are then processed further, that is to say refined. This entails initially for example the plant mucilage and suspended matter being removed. So-called desliming can take place enzymatically or, for example, chemically/physically by adding acid such as phosphoric acid. The free fatty acids are then removed by treatment with a base, for example sodium hydroxide solution. The resulting product is thoroughly washed with water to remove the alkali remaining in the product, and is dried. In order to remove the coloring matters still present in the product, the products are subjected to a bleaching with, for example, bleaching earth or activated carbon. Finally, the product is also deodorized for example with steam.

The PUFAs or LCPUFAs produced by this process are preferably C_{20} and/or C_{22} fatty acid molecules having at least four double bonds in the fatty acid molecule, preferably five or six double bonds. These C_{20} and/or C_{22} fatty acid molecules can be isolated from the plant in the form of an oil, lipid or a free fatty acid. Suitable transgenic plants are for example those mentioned above.

These oils, lipids or fatty acids of the invention comprise, as described above, advantageously 6 to 15% palmitic acid, 1 to 6% stearic acid; 7 - 85% oleic acid; 0.5 to 8% vaccenic acid, 0.1 to 1% arachic acid, 7 to 25% saturated fatty acids, 8 to 85% monounsaturated fatty acids and 60 to 85% polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the plants.

Advantageous polyunsaturated, long-chain fatty acids present in the fatty acid esters or fatty acid mixtures such as phosphatidyl fatty acid esters or triacylglyceride esters are preferably at least 10; 11; 12; 13; 14; 15; 16; 17; 18; 19 or 20% by weight based on the total fatty acid content of eicosapentaenoic acid, based on the total fatty acid content, and/or at least 1; 2; 3; 4; 5 or 6% by weight of docosapentaenoic acid, based on the total fatty acid content, and/or at least 1; 2; 3; preferably at least 4; 5; 6; particularly preferably at least 7 or 8 and most preferably at least 9 or 10% by weight of docosahexaenoic acid, based on the total fatty acid content.

The fatty acid esters or fatty acid mixtures which have been produced by the process of the invention further comprise fatty acids selected from the group of fatty acids erucic acid (13-docosaic acid), sterculic acid (9,10-methylene octadec-9-enonic acid), malvalic acid (8,9-methylene heptadec-8-enonic acid), chaulmoogrinic acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienonoic acid), vernonoic acid (9,10-epoxyoctadec-12-enonoic acid), tarinic acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynonic acid, pyrulic acid (t10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynonic acid, petroselenic acid (cis-6-octadecenonic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid, catalpic acid (9t11t13c-octadecatrienoic acid), eleosteric acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienonic acid). In general, the aforementioned fatty acids are advantageously present only in traces in the fatty acid esters or fatty acid mixtures produced by the process of the invention, meaning that their occurrence, based on the total fatty acid content, is less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, particularly preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very particularly preferably less than 4%, 3%, 2% or 1%. In a further preferred form of the invention the occurrence of these aforementioned fatty acids, based on the total fatty acids, is less than 0.9%; 0.8%; 0.7%; 0.6% or 0.5%, particularly preferably less than 0.4%; 0.3%; 0.2%; 0.1%. The fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise less than 0.1% based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, C23:6$^{\Delta 3,8,12,15,18,21}$).

A further embodiment according to the invention is the use of the oils, the lipids, the fatty acids and/or the fatty acid composition, which are produced by the process of the invention, in feeding stuffs, foodstuffs, cosmetics or pharmaceuticals. The oils, lipids, fatty acids or fatty acid mixtures obtained in the process according to the invention can be used for admixture with other oils, lipids, fatty acids or fatty acid mixtures of animal origin, such as, for example, fish oils, in the manner with which the skilled worker is familiar. These oils, lipids, fatty acids or fatty acid mixtures which are produced in this way and consist of vegetable and

animal components can also be used for the preparation of feeding stuffs, foodstuffs, cosmetics or pharmaceuticals.

The term "oil", "lipid" or "fat" is understood as meaning a fatty acid mixture comprising unsaturated and/or saturated, preferably esterified fatty acid(s). It is preferred that the oil, fat or lipid is high in polyunsaturated free or advantageously esterified fatty acid(s), in particular linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid. Preferably, the amount of unsaturated esterified fatty acids is approximately 30%, with an amount of 50% being especially preferred and an amount of 60%, 70%, 80% or more being most preferred. The amount of the fatty acid can be determined by gas chromatography after converting the fatty acids into the methyl esters by transesterification. The oil, lipid or fat can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and the like. In particular, the amount of the various fatty acids can vary, depending on the starting plant.

As described above, the polyunsaturated fatty acid esters advantageously having three, four, five or six, particularly advantageously having five or six double bonds and which have been prepared in the process advantageously take the form of fatty acid esters, for example, sphingolipid esters, phosphoglyceride esters, lipid esters, glycolipid esters, phospholipid esters, monoacylglycerol esters, diacylglycerol esters, triacylglycerol esters or other fatty acid esters, preference being given to phospholipid esters and/or triacylglycerol esters.

Starting with the polyunsaturated fatty acid esters produced thus in the process according to the invention and advantageously having at least three, four, five or six double bonds, the polyunsaturated fatty acids which are present can be liberated for example via treatment with alkali, for example with aqueous KOH or NaOH, or by acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification with, for example, H_2SO_4. However, the fatty acids can also be liberated directly without the above-described processing steps.

Substrates of the nucleic acid sequences used in the process which encode polypeptides with Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase activity and optionally nucleic acid sequences which encode polypetides having ω3-desaturase and/or Δ4-desaturase

activity, and/or of the further nucleic acids which are used, such as the nucleic acid sequences which encode polypeptides of the fatty acid or lipid metabolism selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously C_{16}-, C_{18}- or C_{20}-fatty acids. Preferably, the fatty acids converted in the process as substrates are converted in the form of their acyl-CoA esters and/or in the form of their phospholipid esters.

To produce the long-chain PUFAs according to the invention, the saturated, monounsaturated C_{16}-fatty acids and/or polyunsaturated C_{18}-fatty acids must first, depending on the substrate, be desaturated and/or elongated or only deaturated by the enzymatic activity of a desaturase and/or elongase and subsequently elongated by at least two carbon atoms by an elongase. After one elongation cycle, this enzyme activity leads either starting from C_{16}-fatty acids to C_{18}-fatty acids or starting from C_{18}-fatty acids to C_{20}-fatty acids, and after two elongation cycles starting from C_{16}-fatty acids leads to C_{20}-fatty acids. The activity of the desaturases or elongases used in the process according to the invention preferably leads to C_{20}- and/or C_{22}-fatty acids, advantageously with at least two or three double bonds in the fatty acid molecule, preferably with four, five or six double bonds, especially preferably to C_{22}-fatty acids with at least five double bonds in the fatty acid molecule. Especially preferred products of the process according to the invention are eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid. The C_{18}-fatty acids with at least two double bonds in the fatty acid can be elongated by the enzymatic activity according to the invention in the form of the free fatty acid or in the form of the esters, such as phospholipids, glycolipids, sphingolipids, phosphoglycerides, monoacylglycerol, diacylglycerol or triacylglycerol.

The preferred biosynthesis site of fatty acids, oils, lipids or fats in the plants which are advantageously used is, for example, generally the seed or cell layers of the seed, so that seed-specific expression of the nucleic acids used in the process makes sense. However, it is obvious that the biosynthesis of fatty acids, oils or lipids need not be limited to the seed tissue, but may also take place in a tissue specific manner in all of the remaining parts of the plant, for example in epidermal cells or in the tubers. The synthesis advantageously takes place according to the inventive process in the vegetative (somatic) tissue.

Owing to the method according to the invention, the polyunsaturated fatty acids which are produced can, in principle, be increased in two ways in the plants used in the process. Advantageously the pool of free polyunsaturated fatty acids and/or the amount of the esterified polyunsaturated fatty acids produced by the process can be increased. Advantageously, the pool of esterified polyunsaturated fatty acids in the transgenic plants is increased by the process according to the invention, advantageously in the form of the phosphatidyl esters and/or triacyl esters.

The sequences used in the process of the invention are cloned singly into expression constructs or provided on a joint recombinant nucleic acid molecule and used for introduction and for expression in organisms. These expression constructs make it possible for the polyunsaturated fatty acids produced in the process of the invention to be synthesized optimally.

The nucleic acids used in the process may, after introduction into a plant or plant cell, either be located on a separate plasmid or advantageously be integrated into the genome of the host cell. In the case of integration into the genome, the integration may be random or take place by recombination such that the native gene is replaced by the introduced copy, thus modulating production of the desired compound by the cell, or through use of a gene in trans, so that the gene is functionally connected to a functional expression unit which comprises at least one sequence ensuring the expression of a gene and at least one sequence ensuring the polyadenylation of a functionally transcribed gene. The nucleic acid sequences are advantageously introduced into the plants via multiexpression cassettes or constructs for multiparallel expression, i.e. the nucleic acid sequences are present in a joint expression unit.

The nucleic acid construct may comprise more than one nucleic acid sequence coding for a polypeptide having the enzymatic activity of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase, and/or ω3-desaturase. It is also possible for a plurality of copies of a nucleic acid sequence coding for a polypeptide having the enzymatic activity of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase, and/or ω3-desaturase to be present.

For the introduction, the nucleic acids used in the process are advantageously amplified and ligated in the known manner. Preferably, a procedure following the protocol for Pfu DNA polymerase or a Pfu/Taq DNA polymerase mixture is followed. The primers are selected depending on the sequence to be amplified. The primers should expediently be chosen in

such a way that the amplicon comprises the entire codogenic sequence from the start codon to the stop codon. After the amplification, the amplificon is expediently analyzed. For example, the analysis can be carried out by gel-electrophoretic separation with respect to quality and quantity. Thereafter, the amplicon can be purified following a standard protol (for example Qiagen). An aliquot of the purified amplicon is then available for the subsequent cloning step. Suitable cloning vectors are generally known to the skilled worker. These include, in particular, vectors which are capable of replication in microbial systems, that is to say mainly vectors which ensure efficient cloning in yeasts or fungi and which make possible the stable transformation of plants. Those which must be mentioned in particular are various binary and cointegrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they comprise at least the vir genes required for the Agrobacterium-mediated transformation and the T-DNA-delimiting sequences (T-DNA border). These vector systems preferably also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers, by means of which suitably transformed organisms can be identified. While in the case of cointegrated vector systems vir genes and T-DNA sequences are arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir genes. Owing to this fact, the last-mentioned vectors are relatively small, easy to manipulate and capable of replication both in E. coli and in Agrobacterium. These binary vectors include vectors from the series pBIB-HYG, pPZP, pBecks, pGreen. In accordance with the invention, pBin19, pBI101, pBinAR, pGPTV and pCAMBIA are used by preference. An overview of the binary vectors and their use is found in Hellens et al. (2000) Trends in Plant Science 5: 446–451.

In order to prepare the vectors, the vectors can first be linearized with restriction endonuclease(s) and then modified enzymatically in a suitable manner. Thereafter, the vector is purified, and an aliquot is employed for the cloning step. In the cloning step, the enzymatically cleaved and, if appropriate, purified amplificate is cloned with vector fragments which have been prepared in a similar manner, using ligase. In this context, a particular nucleic acid construct, or vector or plasmid construct, can have one or more than one codogenic gene segments. The codogenic gene segments in these constructs are preferably linked operably with regulatory sequences. The regulatory sequences include, in particular, plant sequences such as the above-described promoters and terminators. The constructs can advantageously be stably propagated in microorganisms, in particular

Escherichia coli and Agrobacterium tumefaciens, under selective conditions and thus make possible the transfer of heterologous DNA into plants.

The nucleic acid sequences and nucleic acid constructs used in the inventive process can be introduced into microorganisms and then into plants, advantageously using cloning vectors, and thus be used in the transformation of plants such as those which are published in and cited therein: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, p. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. (1991) 42: 205-225. Thus, the nucleic acids, nucleic acid constructs and/or vectors used in the process can be used for the recombinant modification of a broad spectrum of plants so that the latter become better and/or more efficient LCPUFA producers.

Owing to the introduction of a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and Δ5-elongase gene into a plant, alone or in combination with other genes, it is not only possible to increase biosynthesis flux towards the end product, but also to increase, or to create *de novo* the corresponding triacylglycerol and/or phosphatidylester composition. Likewise, the number or activity of other genes which are involved in the import of nutrients which are required for the biosynthesis of one or more fatty acids, oils, polar and/or neutral lipids, can be increased, so that the concentration of these precursors, cofactors or intermediates within the cells or within the storage compartment is increased, whereby the ability of the cells to produce PUFAs, as described below, is enhanced further. By optimizing the activity or increasing the number of one or more of the Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase genes which are involved in the biosynthesis of these compounds, or by destroying the activity of one or more genes which are involved in the degradation of these compounds, it may be possible to increase the yield, production and/or production efficiency in fatty acid and lipid molecules from organisms and advantageously from plants.

The nucleic acid molecules used in the process of the invention code for proteins or parts thereof, whereas the proteins or the individual protein or parts thereof comprises an amino acid sequence which has sufficient homology to an amino acid sequence which is depicted in the sequences SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172 or SEQ ID NO: 52 and, if appropriate, SEQ ID NO: 194 or SEQ ID NO: 78, so that the proteins or parts thereof still

have a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase activity and, if appropriate, a Δ4-desaturase and/or ω3-desaturase activity. The proteins or parts thereof which is/are encoded by the nucleic acid molecule/nucleic acid molecules preferably still have its/their essential enzymatic activity and the ability to participate in the metabolism of compounds necessary for constructing cell membranes or lipid bodies in organisms, advantageously in plants, or in the transport of molecules across these membranes. The proteins encoded by the nucleic acid molecules are at least about 60% and preferably at least about 70%, 80% or 90%, and particularly preferably at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequences depicted in SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78. Homology or homologous means in the context of the invention identity or identical.

The homology was calculated over the entire amino acid or nucleic acid sequence region. To compare various sequences, the skilled worker has available a series of programs which are based on various algorithms. The algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution (1987) 25: 351-360; Higgins et al. (1989) CABIOS 5: 151–153) or the programs Gap and BestFit (Needleman and Wunsch (1970) J. Mol. Biol. 48: 443-453 and Smith and Waterman (1981) Adv. Appl. Math. 2: 482-489), which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used to carry out the sequence comparisons. The sequence homology data given above in % were determined over the entire sequence region using the program GAP with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for sequence comparisons.

Essential enzymatic activity of the ω3-desaturase, Δ6-desaturase, Δ6-elongase, Δ5-elongase, Δ4-desaturase and/or Δ5-desaturase used in the process of the invention means that, compared with the proteins/enzymes encoded by the sequence having SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, they still have an enzymatic activity of at least 10%, preferably of at least 20%, particularly preferably of at least 30% and most preferably of at least 40, 50 or 60%, and thus are able to participate in the metabolism of compounds necessary for synthesizing fatty acids, advantageously fatty

acid esters such as phosphatidyl esters and/or triacylglyceride esters, in a plant or plant cell, or in the transport of molecules across membranes.

Nucleic acids which can be advantageously used in the process are derived from bacteria, fungi, diatoms, animals such as Caernorhabditis or Oncorhynchus or plants such as algae or mosses such as the genera Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Pytium irregulare, Mantoniella, Ostreococcus, Isochrysis, Aleurita, muscarioides, Mortierella, Borago, Phaeodactylum, Crypthecodinium, specifically from the genera and species Pytium irregulare, Oncorhynchus mykiss, Xenopus laevis, Ciona intestinalis, Thalassiosira pseudonona, Mantoniella squamata, Ostreococcus sp., Ostreococcus tauri, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricornutum, Caenorhabditis elegans or particularly advantageously from Pytium irregulare, Thraustochytrium sp. and/or Ostreococcus tauri.

It is possible additionally to use in the process of the invention nucleotide sequences which code for a Δ12-desaturase, Δ9-elongase or Δ8-desaturase. The nucleic acid sequences used in the process are advantageously introduced in an expression cassette which makes expression of the nucleic acids in plants possible.

The nucleic acid sequences which code for the Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase are functionally linked to one or more regulatory signals to increase the gene expression. These regulatory sequences are intended to make targeted expression of the genes possible. This may mean for example, depending on the plant, that the gene is expressed and/or overexpressed only after induction, or that it is expressed and/or overexpressed immediately. Sequences advantageously used for the expression make constitutive expression possible, such as CaMV35S, CaMV36S, CaMV35Smas, nos, mas, ubi, stpt, lea or Super promoter. Expression preferably takes place in vegetative tissue as described above. In another preferred embodiment, the expression takes place in seeds.

These regulatory sequences are for example sequences to which inducers or repressors bind and thus regulate the expression of the nucleic acid. In addition to the regulatory sequences which are not linked in their natural locus to the nucleic acid sequences, or instead of these sequences, the natural regulation of these sequences may still be present before the actual

structural genes and, if appropriate, have been genetically modified so that natural regulation is switched off and expression of the genes is increased. The gene construct may additionally advantageously also comprise one or more so-called "enhancer sequences" functionally linked to the promoter, which make increased expression of the nucleic acid sequence possible. Additional advantageous sequences can also be inserted at the 3' end of the DNA sequences, such as further regulatory elements or terminators. Advantageous terminators are for example viral terminators such as the 35S terminator or others. The nucleic acid sequences used in the process according to the invention may be present in one or more copies of the expression cassette (= gene construct). Preferably, only one copy of the genes is present in each expression cassette. This gene construct, or the gene constructs, can be introduced into the plant simultaneously or successively and expressed together in the host organism. In this context, the gene construct(s) can be inserted in one or more vectors and be present in the cell in free form, or else be inserted in the genome. It is advantageous for the insertion of further genes in the plant when the genes to be expressed are present together in one gene construct. However, it is also possible to introduce in each case one gene construct containing a nucleic acid sequence into a plant and to cross the resulting plants with one another in order to obtain progeny which contains all gene contructs together.

In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the gene expression of the genes introduced, thus enhancing it. Thus, an enhancement of the regulatory elements, advantageously at the transcriptional level, may take place by using strong transcription signals such as promoters and/or enhancers. In addition, however, enhanced translation is also possible, for example by improving the stability of the mRNA.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations, each of the nucleic acids which encode Δ6-desaturase, Δ6-elongase, Δ5-desaturase or Δ5-elongase and if appropriate the ω3-desaturase or Δ4-desaturase and which are used in the process should be expressed under the control of a separate promoter. This can be identical or different for each of the sequences. In this context, the expression cassette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site for insertion of the nucleic acid to be expressed, which cleavage site is advantageously in a polylinker. If appropriate, a terminator can be positioned behind the polylinker. This sequence is repeated several times, preferably three, four, five or six times, so that up to six genes can be combined in one construct and thus introduced into the

transgenic plant in order to be expressed. To express the nucleic acid sequences, the latter are inserted behind the promoter via the suitable cleavage site, for example in the polylinker. Advantageously, each nucleic acid sequence has its own promoter and, if appropriate, its own terminator. However, it is also possible to insert a plurality of nucleic acid sequences behind a promoter and, if appropriate, before a terminator. Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to say a nucleic acid sequence can be inserted at the first or last position in the cassette without the expression being substantially influenced by the position. In an advantageous embodiment, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminators can be used in the expression cassette. In a further advantageous embodiment, identical promoters such as the CaMV35S promoter can also be used.

As described above, the transcription of the genes which have been introduced should advantageously be terminated by suitable terminators at the 3' end of the biosynthesis genes which have been introduced (behind the stop codon). An example of a sequence which can be used in this context is the OCS 1 or the 35SCaMV terminator. As is the case with the promoters, different terminator sequences should be used here for each gene.

As described above, the gene construct can also comprise further genes to be introduced into the organisms. It is possible and advantageous to introduce into the host plants, and to express therein, regulatory genes such as genes for inductors, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a biosynthesis pathway. These genes can be of heterologous or of homologous origin. Moreover, further biosynthesis genes of the fatty acid or lipid metabolism can advantageously be present in the nucleic acid construct, or gene construct or alternatively, these genes can also be present on one further or more further nucleic acid constructs. A biosynthesis gene of the fatty acid or lipid metabolism which is preferably chosen is one or more genes selected from the group of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl- transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) or combinations thereof. Especially advantageous nucleic acid sequences are biosynthesis genes of the fatty acid or lipid

metabolism selected from the group of the acyl-CoA:lysophospholipid acyltransferase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase and/or Δ9-elongase.

In this context, the abovementioned nucleic acids or genes can be cloned into expression cassettes, like those mentioned above, in combination with other elongases and desaturases and used for transforming plants with the aid of Agrobacterium.

The term "vector" used in this description relates to a nucleic acid molecule which is capable of transporting another nucleic acid to which it is bound. One type of vector is a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated. A further type of vector is a viral vector, it being possible for additional DNA segments to be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they have been introduced (for example bacterial vectors with bacterial replication origin). Other vectors are advantageously integrated into the genome of a host cell when they are introduced into the host cell, and thus replicate together with the host genome. Moreover, certain vectors can govern the expression of genes with which they are in operable linkage. These vectors are referred to in the present context as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids. In the present description, "plasmid" and "vector" can be used exchangeably since the plasmid is the form of vector which is most frequently used. However, the invention is also intended to cover other forms of expression vectors, such as viral vectors, which exert similar functions. Furthermore, the term vector is also intended to encompass other vectors with which the skilled worker is familiar, such as phages, viruses such as SV40, CMV, TMV, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA.

The recombinant expression vectors advantageously used in the process comprise the nucleic acid sequences or the above-described gene construct used in the process in a form which is suitable for expressing the nucleic acids used in a host cell, which means that the recombinant expression vectors comprise one or more regulatory sequences, which are selected on the basis of the host cells to be used for the expression, which regulatory sequence(s) is/are linked operably with the nucleic acid sequence to be expressed. In a recombinant expression vector, "linked operably" means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the expression of the nucleotide sequence is made possible and they are bound to each other in such a way that both sequences carry out the predicted function which is ascribed to the sequence (for example in an in-vitro transcription/translation system, or in a host cell if the vector is introduced into the host cell).

The term "regulatory sequence" is intended to comprise promoters, enhancers and other expression control elements (for example polyadenylation signals). These regulatory sequences are described, for example, in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Florida, Ed.: Glick and Thompson, Chapter 7, 89-108, including the references cited therein. Regulatory sequences comprise those which govern the constitutive expression of a nucleotide sequence in many types of host cell and those which govern the direct expression of the nucleotide sequence only in specific host cells under specific conditions. The skilled worker knows that the design of the expression vector can depend on factors such as the choice of host cell to be transformed, the desired degree of expression of the protein and the like.

The recombinant expression vectors used can be designed for the expression of the nucleic acid sequences used in the process in such a way that they can be transformed into prokaryotic intermediate hosts and finally, after introduction into the plants, make expression of the genes possible therein. This is advantageous because on account of simplicity, intermediate steps in vector construction are frequently carried out in microorganisms. For example, the Δ6-desaturates, Δ6-elongase, Δ5-desaturate and/or Δ5-elongase genes can be expressed in bacterial cells, insect cells (using baculovirus expression vectors), yeast cells and other fungal cells (see Romanos, M.A., et al. (1992) Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Editors, pp. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J., & Punt, P.J. (1992) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Editors, pp. 1-28, Cambridge University Press: Cambridge), Algae (Falciatore et al. (1999) Marine Biotechnology.1: (3):239-251), ciliates, with vectors following a transformation process as described in WO 98/01572, and preferably in cells of multicellular plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants" Plant Cell Rep.:538-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter 6/7, pp. 71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer in: Transgenic Plants, vol. 1, Engineering and Utilization, Editors.: Kung and R. Wu, Academic Press (1993), 128-43; Potrykus (1991) Annu. Rev.

Plant Physiol. Plant Molec. Biol. 42: 205-225 (and references cited therein)). Suitable hosts are what are further discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). The recombinant expression vector may alternatively be transcribed and translated in vitro for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes usually takes place with vectors which comprise constitutive or inducible promoters which control the expression of fusion or non-fusion proteins. Typical fusion expression vectors are inter alia pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), of which glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused to the recombinant target protein.

Examples of suitable inducible non-fusion E. coli expression vectors are inter alia pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression by the pTrc vector is based on transcription by host RNA polymerase from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector is based on transcription from a T7-gn10-lac fusion promoter which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ prophage which harbors a T7 gn1 gene under transcription control of the lacUV 5 promoter.

Other vectors suitable in prokaryotic organisms are known to the skilled worker; these vectors are for example in E. coli pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCI, in streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in bacillus pUB110, pC194 or pBD214, in corynebacterium pSA77 or pAJ667.

In a further embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in the yeast S. cerevisiae include pYeDesaturasec1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for constructing vectors suitable for use in other fungi, such as

the filamentous fungi, are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., editors, pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi (J.W. Bennet & L.L. Lasure, Editors, pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are for example pAG-1, YEp6, YEp13 or pEMBLYe23.

Alternatively, the nucleic acid sequences used in the process of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expressing proteins in cultured insect cells (e.g. Sf9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The above mentioned vectors provide only a small survey of possible suitable vectors. Further plasmids are known to the skilled worker and are described for example in: Cloning Vectors (Editors Pouwels, P.H. et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Further suitable expression systems for prokaryotic and eukaryotic cells see in chapters 16 and 17 of Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

The genes used in the process can also be expressed in single-celled plant cells (such as algae), see Falciatore et al. (1999) Marine Biotechnology 1 (3):239-251 and references cited therein, and in plant cells from higher plants (for example spermatophytes such as arable crops). Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) Plant Mol. Biol. 20:1195-1197; and Bevan, M.W. (1984) Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38.

A plant expression cassette preferably comprises regulatory sequences which are capable of governing the expression of genes in plant cells and are linked operably so that each sequence can fulfil its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as gene 3 of the Ti plasmid pTiACH5 (Gielen et al., (1984) EMBO

J. 3 835 et seq.), which is known as octopine synthase, or functional equivalents thereof, but all other terminators which are functionally active in plants are also suitable.

Since the regulation of plant gene expression is very often not limited to the transcriptional level, a plant expression cassette preferably comprises other sequences which are linked operably, such as translation enhancers, for example the overdrive sequence, which enhances the tobacco mosaic virus 5' - untranslated leader sequence, which increases the protein/RNA ratio (Gallie et al. (1987) Nucl. Acids Research 15:8693-8711).

As described above, the plant gene expression must be linked operably with a suitable promoter which controls gene expression. Advantageously utilizable promoters are constitutive promoters (Benfey et al., EMBO J. (1989) 8: 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al. (1980) Cell 21: 285-294), 19S CaMV (see also US 5352605 and WO 84/02913), or plant promoters, such as the promoter of the Rubisco small subunit, which is described in US 4,962,028.

Other preferred sequences for use for functional connection in plant gene expression cassettes are targeting sequences which are necessary for guiding the gene product into its appropriate cellular compartment, for example into the vacuoles, the cell nucleus, all types of plastids such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells; (see a review in Kermode (1996) Crit. Rev. Plant Sci. 15(4): 284-423 and literature cited therein).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via traditional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction as used in the present context are intended to encompass the multiplicity of prior-art methods for introducing heterologous nucleic acids (for example DNA) into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed: Gartland and Davey, Humana Press, Totowa, New Jersey.

The term "nucleic acid (molecule)" as used herein comprises in an advantageous embodiment

additionally the untranslated sequence located at the 3' end and at the 5' end of the coding gene region: at least 500, preferably 200, particularly preferably 100 nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, particularly preferably 20 nucleotides of the sequence downstream of the 3' end of the coding gene region. An "isolated" nucleic acid molecule is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably has no sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid is derived (e.g. sequences located at the 5' and 3' ends of the nucleic acid). In various embodiments, the isolated Δ6-desaturase, Δ6-elongase or Δ5-desaturase and, if appropriate, the ω3-desaturase or Δ4-desaturase molecule used in the process may for example comprise less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived.

The nucleic acid molecules used in the process can be isolated by using standard techniques of molecular biology and the sequence information provided herein. It is also possible for example with the aid of comparative algorithms to identify a homologous sequence or homologous, conserved sequence regions at the DNA or amino acid level. These can be used as hybridization probe in standard hybridization techniques (as described for example in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for isolating further nucleic acid sequences useful in the process. The nucleic acid molecule used in the process, or parts thereof, can moreover be isolated by polymerase chain reaction, in which case oligonucleotide primers based on this sequence or on parts thereof are used (e.g. a nucleic acid molecule comprising the complete sequence or a part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been constructed on the basis of this identical sequence). For example, mRNA can be isolated from cells (e.g. by the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA can be prepared with the aid of reverse transcriptase (e.g. Moloney MLV reverse transcriptase obtainable from Gibco/BRL, Bethesda, MD or AMV reverse transcriptase, obtainable from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligo-nucleotide primers for amplification by means of polymerase chain reaction can be constructed on the basis of one of the sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 or with the aid of the

amino acid sequences depicted in SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78. A nucleic acid of the invention can be amplified by standard PCR amplification techniques using cDNA or alternatively genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified in this way can be cloned into a suitable vector and characterized by DNA sequence analysis. Oligonucleotides can be prepared by standard synthetic methods, for example using an automatic DNA synthesizer.

Homologs of the Δ5-elongase, ω3-desaturase, Δ6-desaturase, Δ6-elongase, Δ4-desaturase or Δ5-desaturase nucleic acid sequences used, having the sequence SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, mean for example allelic variants having at least about 40, 50 or 60%, preferably at least about 60 or 70%, more preferably at least about 70 or 80%, 90% or 95% and even more preferably at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity or homology to one of the nucleotide sequences shown in SEQ ID NO: 64, 66, 68 or 70, to one of the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41, to one of the nucleotide sequences shown in SEQ ID NO: 171, 173, 175, 177, 179, 181 or 183, to one of the nucleotide sequences shown in SEQ ID NO: 51, 53 or 55, to one of the nucleotide sequences shown in SEQ ID NO: 193 or 195 or to one of the nucleotide sequences shown in or SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, especially the nucleotide sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, or their homologs, derivatives or analogs or parts thereof. Also included are isolated nucleic acid molecules of a nucleotide sequence which hybridize for example under stringent conditions to one of the nucleotide sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 or a part thereof. A part means in this connection according to the invention that at least 25 base pairs (= bp), 50 bp, 75 bp, 100 bp, 125 bp or 150 bp, preferably at least 175 bp, 200 bp, 225 bp, 250 bp, 275 bp or 300 bp, particularly preferably 350 bp, 400 bp, 450 bp, 500 bp or more base pairs are used for the hybridization. It is also possible advantageously to use the complete sequence. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from the sequence depicted in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, but where the enzyme activity of the proteins encoded thereby is substantially retained for the

insertion.

Nucleic acid molecules advantageous for the process of the invention can be isolated on the basis of their homology to the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase and/or Δ6-elongase nucleic acid sequences disclosed herein by using the sequences or a part thereof as hybridization probe in standard hybridization techniques under stringent hybridization conditions. It is possible in this connection for example to use isolated nucleic acid molecules which are at least 15 nucleotides long and hybridize under stringent conditions with the nucleic acid molecules which comprise a nucleotide sequence of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77. It is also possible to use nucleic acid molecules having at least 25, 50, 100, 250 or more nucleotides.

The term "hybridizes under stringent conditions" as used herein is intended to describe hybridization and washing conditions under which nucleic acid sequences which are at least 60% mutually homologous normally remain hybridized together. The conditions are preferably such that sequences which are at least about 65%, preferably at least about 70% and particularly preferably at least about 75% or more mutually homologous normally remain hybridized together. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-restrictive example of stringent hybridization conditions are hybridizations in 6 x sodium chloride/sodium citrate (= SSC) at about 45°C, followed by one or more washing steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker is aware that these hybridization conditions differ depending on the type of nucleic acid and, for example organic solvents are present, in relation to the temperature and the concentration of the buffer. The temperature for example under "standard hybridization conditions" is, depending on the type of nucleic acid, between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvent, for example 50% formamide, is present in the abovementioned buffer, the temperature under standard conditions is about 42°C. The hybridization conditions for DNA:DNA hybrids are preferably for example 0.1 x SSC and 20°C to 45°C, preferably 30°C to 45°C. The hybridization conditions for DNA:RNA hybrids are preferably for example 0.1 x SSC and 30°C to 55°C, preferably 45°C to 55°C. The aforementioned hybridization temperatures are determined for example for a nucleic acid with a length of about 100 bp (= base pairs) and a G + C content of 50% in the absence of formamide. The skilled person knows how the necessary hybridization conditions

can be determined on the basis of textbooks such as the abovementioned or from the following textbooks Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (editors) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (editor) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

In order to determine the percentage of homology (= identity) of two amino acid sequences (for example one of the sequences of SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78) or of two nucleic acids (for example SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77), the sequences are written one under the other in order to be able to compare them optimally (for example, gaps may be introduced into the sequence of a protein or of a nucleic acid in order to generate optimal alignment with the other protein or the other nucleic acid). Then, the amino acid radicals or nucleotides at the corresponding amino acid positions or nucleotide positions are compared. If a position in a sequence is occupied by the same amino acid radical or the same nucleotide as the corresponding position in another sequence, then the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity"). The percentage of homology between the two sequences is a function of the number of identical positions which the sequences share (i.e. % homology = number of identical positions/total number of positions x 100). The programs and algorithms used to determine the homology are described above.

An isolated nucleic acid molecule which codes for an ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase and/or Δ6-elongase which is used in the process and which is homologous to a protein sequence of SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78 can be generated by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, so that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations may be introduced into one of the sequences of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 by standard techniques such as site-specific mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are preferably produced at one or more of the predicted nonessential amino acid residues. In a "conservative amino acid

substitution" the amino acid residue is replaced by an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid residue in an ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase or Δ6-elongase is thus preferably replaced by another amino acid residue from the same side-chain family. An alternative possibility in another embodiment is to introduce the mutations randomly over the whole or a part of the ω3-desaturase-, Δ6-desaturase-, Δ5-desaturase-, Δ5-elongase-, Δ4-desaturase- or Δ6-elongase-encoding sequence, e.g. by saturation mutagenesis, and the resulting mutants can be screened for the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase or Δ6-elongase activity described herein in order to identify mutants which have retained the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase,Δ4-desaturase or Δ6-elongase activity. The encoded protein can be recombinantly expressed after the mutagenesis, and the activity of the protein can be determined for example by using the assays described herein.

The invention is illustrated in greater detail by the examples which follow, which are not to be construed as limiting. The content of all of the references, patent applications, patents and published patent applications cited in the present patent application is herewith incorporated by reference.

The following table shows the sequence identifiers as used in the priority application of February 21, 2006, with the German application number 102006008030.0, and the corresponding sequence identifiers in this subsequent application. The nucleic acid sequence identified by SEQ ID NO: 1 of the priority application corresponds for example to the nucleic acid sequence identified by SEQ ID NO: 64 of the subsequent application.

Table of concordance of sequence identifiers of the priority application and the sequence identifiers in the subsequent application:

SEQ ID NO: Priority application	**SEQ ID NO:**	**Organism**

German application number 102006008030.0	this subsequent application	
1	64	Ostreococcus tauri
2	65	Ostreococcus tauri
3	1	Phytium irregulare
4	2	Phytium irregulare
5	171	Traustochytrium sp.
6	172	Traustochytrium sp.
7	51	Thraustochytrium ssp.
8	52	Thraustochytrium ssp.
9	193	Phytophthora infestans
10	194	Phytophthora infestans
11	77	Traustochytrium sp.
12	78	Traustochytrium sp.
13	109	Ostreococcus tauri
n.a.	110	Ostreococcus tauri
14	122	Ostreococcus tauri
n.a.	123	Ostreococcus tauri
15	143	Ostreococcus tauri
16	144	Ostreococcus tauri
17	161	Cauliflower mosaic virus
18	162	Cauliflower mosaic virus
19	163	Thalassiosira pseudonana
20	164	Thalassiosira pseudonana

Examples

Example 1: General cloning methods

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of Escherichia coli cells, bacterial cultures and the sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

Example 2: Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced with an ABI laser fluorescence DNA sequencer by the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74: 5463-5467). Fragments resulting from a polymerase chain reaction were sequenced and verified to avoid polymerase errors in constructs to be expressed.

Example 3: Cloning of genes from Ostreococcus tauri

It was possible by searching for conserved regions in an Ostreococcus tauri sequence database (genomic sequences) in each case a sequence coding for a protein having Δ5-elongase activity or Δ6-elongase activity. These are the following sequences:

Gene name	SEQ ID	Amino acids
OtELO1.1, (Δ6-Elongase)	SEQ ID NO: 143	292
OtELO2.1, (Δ5-Elongase)	SEQ ID NO: 109	300

OtElo2.1 shows greatest similarity to an elongase from Danio rerio (GenBank AAN77156; approx. 26% identity), whereas OtElo1.1 shows greatest similarity to the elongase from Physcomitrella (PSE) (approx. 36% identity) (alignments were carried out with the tBLASTn algorithm (Altschul et al. (1990) J. Mol. Biol. 215: 403-410)).

The cloning of the elongases was carried out as follows:

40 ml of an Ostreococcus tauri culture in the stationary phase were spun down and resuspended in 100 µl of double-distilled water and stored at -20°C. The corresponding genomic DNAs were amplified by the PCR method. The corresponding primer pairs were selected so that they harbored the yeast consensus sequence for high-efficiency translation (Kozak (1986) Cell 44: 283-292) beside the start codon. Amplification of the OtElo DNAs was carried out in each case with 1 µl of thawed cells, 200 µM dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 µl. The conditions for the PCR were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes.

Example 4: Optimization of elongase genes from Ostreococcus tauri

Elongases from the organism Ostreococcus tauri were isolated as described in example 3. In

order to achieve an increase in the content of C22 fatty acids, the sequences SEQ ID NO: 143 (Δ6-elongase) and SEQ ID NO: 109 (coding for a protein identified by SEQ ID NO: 110) (Δ5-elongase) were adapted to the codon usage in oilseed rape, flax and soybean. For this purpose, the amino acid sequence of the Δ6-elongase and of the Δ5-elongase (SEQ ID NO: 144 for the Δ6-elongase; SEQ ID NO: 65 for the Δ5-elongase) was back-translated to obtain degenerate DNA sequences. These DNA sequences were adapted by means of the GeneOptimizer program (from Geneart, Regensburg) to the codon usage in oilseed rape, soybean and flax, taking account of the natural frequency of individual codons. The optimized sequences obtained in this way, which are indicated in SEQ ID NO: 64 (Δ5-elongase) and SEQ ID NO: 122 (coding for a protein identified by SEQ ID NO: 123) (Δ6-elongase) were synthesized in vitro.

Example 5: Cloning of expression plasmids for heterologous expression in yeasts

To characterize the function of the optimized nucleic acid sequences, the open reading frames of the respective DNAs were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), resulting in the plasmids pOTE1.2 (comprising the Δ6-elongase sequence) and pOTE2.2 (comprising the Δ5-elongase sequence).

Overview of the elongase sequences cloned into the yeast vector pYES2.1/V5-His-TOPO:

Gene name	SEQ ID	Amino acids
pOTE1.1, (Δ6-elongase)	SEQ ID NO: 143	292
pOTE1.2, (Δ6-elongase)	SEQ ID NO: 122	292, codon-optimized
pOTE2.1, (Δ5-elongase)	SEQ ID NO: 109	300
pOTE2.2, (Δ5-elongase)	SEQ ID NO: 64	300, codon-optimized

The Saccharomyces cerevisiae strain 334 was transformed by electroporation (1500 V) with the vectors pOTE1.2 and pOTE2.2 and with the comparative constructs pOTE1.1 and pOTE2.1 which comprise the natural nucleic acid sequence coding for the Δ6-elongase and Δ5-elongase, respectively. A yeast transformed with the empty vector pYES2 was used as control. The transformed yeasts were selected on complete minimal medium (CMdum) agar plates with 2% glucose but without uracil. After the selection, three transformants in each case were selected for further functional expression.

To express the Ot elongases, initially precultures composed of in each case 5 ml of CMdum

liquid medium with 2% (w/v) raffinose but without uracil were inoculated with the selected transformants and incubated at 30°C, 200 rpm for 2 days. 5 ml of CMdum liquid medium (without uracil) with 2% raffinose were then inoculated with the precultures to an OD_{600} of 0.05. Moreover, 0.2 mM γ-linolenic acid (GLA) was added in each case to the yeast culture transformed with pOTE1.1 and pOTE1.2. On the basis of the activity of OtELO1.1, an elongation of the γ-linolenic acid to the 20:3 fatty acid is to be expected. Respectively 0.2 mM arachidonic acid and eicosapentaenoic acid were added in each case to the yeast culture transformed with pOTE2.1 and pOTE2.2. Corresponding to the activity of OtELO2.1, it is to be expected that the fatty acids ARA and EPA will be elongated respectively to the 22:4 and 22:5 fatty acids. Expression was induced by adding 2% (w/v) galactose. The cultures were incubated at 20°C for a further 96 h.

Example 6: Expression of OtELO2.2 (as depicted in SEQ ID NO: 64) and OtELO1.2 (as in SEQ ID NO: 122) in yeasts

Yeasts transformed as in example 5 with the plasmids pYES2, pOTE1.2 and pOTE2.1 were analyzed in the following way:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0, in order to remove remaining medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acidic methanolysis. For this purpose, the cell sediments were incubated with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) dimethoxypropane at 80°C for 1 h. The FAMES were extracted by extraction twice with petroleum ether (PE). To remove underivatized fatty acids, the organic phases were washed once each with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and with 2 ml of distilled water. The PE phases were then dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C at a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with appropriate fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson (2001) Lipids 36(8): 761-766; Sayanova et al. (2001) Journal of Experimental Botany 52(360): 1581-1585, Sperling et al. (2001) Arch. Biochem. Biophys. 388(2): 293-298 and

Michaelson et al. (1998) FEBS Letters 439(3): 215-218. The results of the analyses are depicted in table 1.

It was possible to confirm the appropriate activities both for pOTE1.1/pOTE1.2 and for pOTE2.1/2.2. The optimized sequence (respectively pOTE1.2 and pOTE2.2) showed activity in both cases. Synthesis of γ-linolenic acid could be increased only slightly by pOTE1.2 compared with the wild-type sequence. By contrast, it was possible to observe for pOTE2.2 surprisingly both an increase in the activity and an alteration in the specificity (table 1). In this connection, the activity for elongation of EPA had virtually doubled, while the elongation of ARA had more than quadrupled. It was thus possible with the optimization of the sequence of the Δ5-elongase from Ostreococcus tauri to increase the yield of the precursors of DHA 6-fold in yeast with the same amount of substrate.

Example 7: Cloning expression plasmids for the seed-specific expression in plants

The following general conditins described apply to all subsequent experiments unless described otherwise.

pBin19, pBI101, pBinAR, pGPTV, pCAMBIA or pSUN are preferably used for the following examples in accordance with the invention. An overview of the binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5: 446-451. A pGPTV derivative as described in DE10205607 was used. This vector differs from pGPTV by an additionally inserted AscI restriction cleavage site.

Starting point of the cloning procedure was the cloning vector pUC19 (Maniatis et al.). In the first step, the conlinin promoter fragment was amplified using the following primers:

Cnl1 C 5': gaattcggcgcgccgagctcctcgagcaacggttccggcggtatagagttgggtaattcga (SEQ ID NO: 200)

Cnl1 C 3': cccgggatcgatgccggcagatctccaccatttttttggtggtgat (SEQ ID NO: 201)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme EcoRI and then for 12 hours at 25°C with the restriction enzyme SmaI. The cloning vector pUC19 was incubated in the same manner. Thereafter, the PCR product and the 2668 bp cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C was verified by sequencing.

In the next step, the OCS terminator (Genbank Accession V00088; De Greve, H., et al. (1982) J. Mol. Appl. Genet. 1 (6): 499-511) was amplified from the vector pGPVT-USP/OCS (DE 102 05 607) using the following primers:

OCS_C 5': aggcctccatggcctgctttaatgagatatgcgagacgcc (SEQ ID NO: 202)

OCS_C 3': cccgggccggacaatcagtaaattgaacggag (SEQ ID NO: 203)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25°C with the restriction enzyme SmaI. The vector pUC19-Cn11-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_OCS was verified by sequencing.

In the next step, the Cnl1-B promoter was amplified by PCR by means of the following primers:

Cnl1-B 5': aggcctcaacggttccggcggtatag (SEQ ID NO: 204)

Cnl1-B 3': cccggggttaacgctagcgggcccgatatcggatcccatttttggtggtgattggttct (SEQ ID NO: 205)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25°C with the restriction enzyme SmaI. The vector pUC19-Cn11-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cn11B_OCS was verified by sequencing.

In a further step, the OCS terminator for Cnl1B was inserted. To this end, the PCR was carried out using the following primers:

OCS2 5': aggcctcctgctttaatgagatatgcgagac (SEQ ID NO: 206)

OCS2 3': cccgggcggacaatcagtaaattgaacggag (SEQ ID NO: 207)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25°C with the restriction enzyme SmaI. The vector pUC19-Cnl1C_Cnl1B_OCS was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cnl1B_OCS2 was verified by sequencing.

In the next step, the Cnl1-A promoter is amplified by PCR using the following primers:

Cnl1-B 5': aggcctcaacggttccggcggtatagag (SEQ ID NO: 208)

Cnl1-B 3': aggccttctagactgcaggcggccgcccgcatttttttggtggtgattggt (SEQ ID NO: 209)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme StuI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was verified by sequencing.

In a further step, the OCS terminator for Cnl1A was inserted. To this end, the PCR was carried out with the following primers:

OCS2 5': ggcctcctgctttaatgagatatgcga (SEQ ID NO: 210)

OCS2 3': aagcttggcgcgccgagctcgtcgacggacaatcagtaaattgaacggaga (SEQ ID NO: 211)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 2 hours at 37°C with the restriction enzyme HindIII. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was incubated for 2 hours at 37°C with the restriction enzyme StuI and for 2 hours at 37°C with the restriction enzyme HindIII. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cnl1B_Cnl1A_OCS3 was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the Δ6-, Δ5-desaturase and Δ6-elongase. To this end, the Phytium irregulare Δ6-desaturase (WO02/26946) was amplified using the following PCR primers:

D6Des(Pir) 5': agatctatggtggacctcaagcctggagtg (SEQ ID NO: 212)

D6Des(Pir) 3': ccatggcccgggttacatcgctgggaactcggtgat (SEQ ID NO: 213)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and then for 2 hours at 37°C with the restriction enzyme NcoI. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and for 2 hours at 37°C with the restriction enzyme NcoI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir) was used for cloning the Thraustochytrium ssp. Δ5-desaturase (WO02/26946). To this end, the Thraustochytrium ssp. Δ5-desaturase was amplified using the following PCR primers:

D5Des(Tc) 5': gggatccatgggcaagggcagcgagggccg (SEQ ID NO: 214)

D5Des(Tc) 3': ggcgccgacaccaagaagcaggactgagatatc (SEQ ID NO: 215)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme BamHI and then for 2 hours at 37°C with the restriction enzyme *Eco*RV. The vector pUC19-Cnl1_d6Des(Pir) was incubated for 2 hours at 37°C with the restriction enzyme BamHI and for 2 hours at 37°C with the restriction enzyme EcoRV. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was used for cloning the Physcomitrella patens Δ6-elongase (WO01/59128), for which purpose the latter was amplified using the following PCR primers:

D6Elo(Pp) 5': gcggccgcatggaggtcgtggagagattctacggtg (SEQ ID NO: 216)

D6Elo(Pp) 3': gcaaaagggagctaaaactgagtgatctaga (SEQ ID NO: 217)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme NotI and then for 2 hours at 37°C with the restriction enzyme XbaI. The vector pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was incubated for 2 hours at 37°C with the restriction enzyme NotI and for 2 hours at 37°C with the restriction enzyme XbaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was verified by sequencing.

The binary vector for the transformation of plants was prepared starting from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp). To this end, pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was incubated for 2 hours at 37°C with the restriction enzyme AscI. The vector pGPTV was treated in the same manner. Thereafter, the fragment from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) and the cleaved pGPTV vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was verified by sequencing.

A further construct, pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co), was used. To this end, the amplification was carried out with the following primers, starting from pUC19-Cnl1C_OCS:

Cnl1_OCS 5': gtcgatcaacggttccggcggtatagagttg (SEQ ID NO: 218)

Cnl1_OCS 3': gtcgatcggacaatcagtaaattgaacggaga (SEQ ID NO: 219)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme SalI. The vector pUC19 was incubated for 2 hours at 37°C with the restriction enzyme SalI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_OCS was verified by sequencing.

In a further step, the Calendula officinalis Δ12-desaturase gene (WO01/85968) was cloned into pUC19-Cnl1_OCS. To this end, d12Des(Co) was amplified with the following primers:

D12Des(Co) 5': agatctatgggtgcaggcggtcgaatgc (SEQ ID NO: 220)

D12Des(Co) 3': ccatggttaaatcttattacgatacc (SEQ ID NO: 221)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and thereafter for 2 hours at the same temperature with NcoI. The vector pUC19-Cnl1_OCS was incubated in the same manner. Thereafter, the PCR fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_D12Des(Co) was verified by sequencing.

The plasmid pUC19-Cnl1_D12Des(Co) and the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) were incubated for 2 hours at 37°C with the restriction enzyme SalI. Thereafter, the vector fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and vector fragment were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was verified by sequencing.

The binary vector for the transformation of plants was prepared starting from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co). To this end, pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was incubated for 2 hours at 37°C with the restriction enzyme *Asc*I. The vector pGPTV was treated in the same manner. Thereafter, the fragment from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) and the cleaved pGPTV vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The

resulting plasmid pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was verified by sequencing.

A further example of the use of seed-specific expression constructs is the Napin promoter. Preparation of these expression constructs in the vectors pGPTV or pSUN is described in Wu et al. (2005) Nat. Biotech. 23:1013-1017.

A further vector suitable for plant transformation is pSUN2. This vector was used in combination with the Gateway system (Invitrogen, Karlsruhe) in order to increase the number of expression cassettes present in the vector to more than four. For this purpose, the Gateway cassette A was inserted into the vector pSUN2 in accordance with the manufacturer's instructions, as described below:

The pSUN2 vector (1 µg) was incubated with the restriction enzyme EcoRV at 37° for 1 h. The Gateway cassette A (Invitrogen, Karlsruhe) was then ligated into the cut vector using the Rapid Ligation kit from Roche, Mannheim. The resulting plasmid was transformed into E. coli DB3.1 cells (Invitrogen). The isolated plasmid pSUN-GW was then verified by sequencing.

In the second step, the expression cassette was cut out of pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) using AscI and ligated into the likewise treated vector pSUN-GW. The plasmid obtained in this way pSUN-4G was used for further gene constructs.

For this purpose, firstly a pENTR clone was modified in accordance with the manufacturer's instructions (Invitrogen). The plasmid pENTR1A (Invitrogen) was incubated with the restriction enzyme EcoRI at 37° for 1 h and then treated with Klenow enzyme and with a 1 µM dNTP mix for 30 min, and subsequently the AscI adapter (5'-ggcgcgcc; phosphorylated at the 5' end, double-stranded) was ligated into the pENTR1A vector. Genes were inserted as described above stepwise into the Cnl cassette in these modified and transferred via AscI into the pENTR vector, resulting in the pENTR-Cnl vector.

In a further step, the pSUN-8G construct was prepared. For this purpose, 5' and 3' primers for the genes with the SEQ ID NOs: 1, 3, 5 and 7 with the restriction cleavage sites described above and with the first and in each case last 20 nucleotides of the open reading frame were produced and amplified with the standard conditions (see above) and ligated into the pENTR-Cnl vector, which was subsequently subjected to a recombination reaction with the pSUN-4G vector in accordance with the manufacturer's instructions.

The construct pSUN-8G was prepared in this way and was transformed into Brassica juncea and Brassica napus. The seeds of the transgenic plants were analyzed by gas chromatography.

A further construct which was used for transformation of B. juncea and B. napus was the construct pSUN-9G. This construct was prepared according to Wu et al. (2005) Nat. Biotech. 23:1013-1017 with the napin promoter. In a modification of Wu et al. 2005, the coding sequence of OtELO2.2 was inserted in the described manner instead of the gene OmELO. The resulting construct pSUN-9G was then transformed into B. juncea and B. napus.

Example 8: Lipid extraction from plant material

The effect of the genetic modification in plants on the production of a desired compound (such as a fatty acid) can be determined by growing the modified plant under suitable conditions (such as those described above) and analyzing the medium and/or the cellular components for the elevated production of the desired product (i.e. of the lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various types of staining methods, enzymatic and microbiological methods and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon A. et al. (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", p. 469-714, VCH: Weinheim; Belter, P.A. et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., and Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., and Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. B3; Chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned methods, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940 and Browse et al. (1986) Analytic Biochemistry 152:141-145. The qualitative and quantitative analysis of lipids or fatty acids is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford:

Pergamon Press, 1 (1952) - 16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

In addition to measuring the end product of the fermentation, it is also possible to analyze other components of the metabolic pathways which are used for the production of the desired compound, such as intermediates and by-products, in order to determine the overall production efficiency of the compound. The analytical methods comprise measuring the amount of nutrients in the medium (for example sugars, hydrocarbons, nitrogen sources, phosphate and other ions), measuring the biomass composition and the growth, analyzing the production of conventional metabolytes of biosynthetic pathways and measuring gases which are generated during the fermentation. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, Ed., IRL Press, p. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAME, fatty acid methyl ester; GC-MS, gas liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

The unambiguous detection for the presence of fatty acid products can be obtained by analyzing recombinant organisms using analytical standard methods: GC, GC-MS or TLC, as described on several occasions by Christie and the references therein (1997, in: Advances on Lipid Methodology, Fourth Edition: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren [Gas chromatography/mass spectrometric methods], Lipide 33:343-353).

The material to be analyzed can be disrupted by sonication, grinding in a glass mill, liquid nitrogen and grinding or via other applicable methods. After disruption, the material must be centrifuged. The sediment is resuspended in distilled water, heated for 10 minutes at 100°C, cooled on ice and recentrifuged, followed by extraction for one hour at 90°C in 0.5 M sulfuric acid in methanol with 2% dimethoxypropane, which leads to hydrolyzed oil and lipid compounds, which give transmethylated lipids. These fatty acid methyl esters are extracted in petroleum ether and finally subjected to a GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) at a temperature gradient of between 170°C and 240°C for 20 minutes and 5 minutes at 240°C. The identity of the resulting fatty

acid methyl esters must be defined using standards which are available from commercial sources (i.e. Sigma).

Plant material is initially homogenized mechanically by comminuting in a pestle and mortar to make it more amenable to extraction.

This is followed by heating at 100°C for 10 minutes and, after cooling on ice, by resedimentation. The cell sediment is hydrolyzed for one hour at 90°C with 1 M methanolic sulfuric acid and 2% dimethoxypropane, and the lipids are transmethylated. The resulting fatty acid methyl esters (FAMEs) are extracted in petroleum ether. The extracted FAMEs are analyzed by gas liquid chromatography using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) and a temperature gradient of from 170°C to 240°C in 20 minutes and 5 minutes at 240°C. The identity of the fatty acid methyl esters is confirmed by comparison with corresponding FAME standards (Sigma). The identity and position of the double bond can be analyzed further by suitable chemical derivatization of the FAME mixtures, for example to give 4,4-dimethoxyazolin derivatives (Christie, 1998) by means of GC-MS.

Example 9: Use of the optimized Δ5-elongase (as depicted in SEQ ID NO: 64) from Ostreococcus tauri for constructs for constitutive expression

Transformation vectors based on pGPTV-35S, a plasmid based on pBIN19-35S (Bevan M. (1984) Nucl. Acids Res. 18:203), were produced for the transformation of plants. For this purpose, firstly an expression cassette consisting of the promoter element CaMV35S (SEQ ID NO: 161) and the 35S terminator (SEQ ID NO: 162; Franck, A. et al. (1980) Cell 21 (1): 285-294) was assembled in a pUC vector. This entailed the promoter being inserted via the SalI/XbaI restriction cleavage sites and the terminator via the BamHI/SmaI restriction cleavage sites. In addition, a polylinker with the XhoI cleavage site was attached to the terminator ('triple ligation'). The resulting plasmid pUC19-35S was then employed for cloning PUFA genes. In parallel, the open reading frames of the Δ6-desaturase (SEQ ID NO: 1), of the Δ5-desaturase (SEQ ID NO: 51) and Δ6-elongase (SEQ ID NO: 171) sequences were inserted via the EcoRV cleavage site into pUC19-35S vectors. The resulting plasmids pUC-D6, pUC-D5, pUC-E6(Tc) were used to construct the binary vector pGPTV-35S_D6D5E6(Tc). For this purpose, the vector pGPTV was digested with the enzyme SalI, the plasmid pUC-D6 was digested with SalI/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6 was then digested with SalI, the plasmid pUC-D5 was

digested with SalI/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6-D5 was then digested once more with SalI, the plasmid pUC-E6(Tc) with SalI/XhoI, and the correct fragments were ligated. These sequential cloning steps resulted in the binary vector pGPTV-D6D5E6(Tc), which was employed for the transformation.

In a further procedure, the sequence of d6Elo(Tp) (SEQ ID NO: 163) was inserted into the vector pUC19-35S instead of the sequence d6Elo(Tc). The resulting plasmid pUC-E6(Tp) was used to prepare the binary vector pGPTV-35S_D6D5E6(Tp).

In a further procedure, the open reading frame of ω3Des (SEQ ID NO: 193) was cloned into pUC19-35S. The resulting plasmid pUC-ω3Pi was transferred via SalI/XhoI into the binary vectors pGPTV-D6D5E6(Tc) and pGPTV-D6D5E6(Tp). The resulting vectors pGPTV-D6D5E6(Tc)ω3Pi and pGPTV-D6D5E6(Tp)ω3Pi were employed for the plant transformation.

In a further procedure, the open reading frame of the optimized Δ5-elongase from Ostreococcus tauri (SEQ ID NO: 64) and the open reading frame of the Δ4-desaturase from Thraustochytrium sp. (SEQ ID NO: 77) was cloned into pUC19-35S. The resulting plasmids pUC-E5 and pUC-D4 were then transferred via SalI/XhoI in accordance with the above statements into the vector pGPTV-D6D5E6(Tp)ω3Pi. The resulting vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was employed for the plant transformation.

All the binary vectors were transformed into E. coli DH5α cells (Invitrogen) in accordance with the manufacturer's instructions. Positive clones were identified by PCR, and plasmid DNA was isolated (Qiagen Dneasy).

Example 10: Transformation of the constitutive binary vectors into plants

a) Generation of transgenic Brassica napus and Brassica juncea plants. The protocol for the transformation of oilseed rape plant was used (modification of Moloney et al. (1992) Plant Cell Reports 8:238-242)

The binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed in Agrobacterium tumefaciens C58C1:pGV2260 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Murashige and Skoog (1962) Physiol. Plant. 15: 473) supplemented with 3% sucrose (3MS medium) was used for the transformation of Orychophragmus violaceus. Petioles or hypocotyls of freshly germinated sterile plants (in

each case approx. 1 cm^2) were incubated with a 1:50 agrobacterial dilution for 5-10 minutes in a Petri dish. This is followed by 3 days of coincubation in the dark at 25°C on 3MS medium supplemented with 0.8% Bacto agar. Thereafter, the cultivation was continued with 16 hours light/8 hours dark and a weekly rhythm on MS medium supplemented with 500 mg/l Claforan (cefotaxime-sodium), 50 mg/l kanamycin, 20 µM benzylaminopurine (BAP) and 1.6 g/l glucose. Growing shoots were transferred to MS medium supplemented with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan, then, after rooting, transferred into soil and, after cultivation, grown for two weeks in a controlled-environment cabinet or in the greenhouse, allowed to flower, mature seeds were harvested and analyzed for elongase expression such as Δ6-elongase activity or for Δ5- or Δ6-desaturase activity by means of lipid analyses. In this manner, lines with elevated contents of polyunsaturated C20- and C22-fatty acids were identified.

b) Generation of transgenic Orychophragmus violaceus plants

The protocol for the transformation of oilseed rape plants was used (modification of Moloney et al. (1992) Plant Cell Reports 8:238-242) as described under a).

To generate transgenic plants, the binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed into Agrobacterium tumefaciens C58C1:pGV2260 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed Agrobacterium colony in Murashige-Skoog medium (Murashige and Skoog (1962) Physiol. Plant, 15: 473) with 3% sucrose (3MS medium) was used to transform Orychophragmus violaceus. Petioles or hypocotyls of freshly germinated sterile plants (each about 1 cm^2) were incubated with a 1:50 agrobacterial dilution in a Petri dish for 5-10 minutes. This is followed by coincubation on 3MS medium with 0.8% Bacto agar in the dark at 25°C for 3 days. The cultivation was then continued with 16 hours light/8 hours dark and in a weekly rhythm on MS medium with 500 mg/l Claforan (cefotaxime sodium), 15 mg/l kanamycin, 20 µM benzylaminopurine (BAP) and 1.6 g/l glucose. Growing shoots were transferred to MS medium with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan and, after rooting, transferred to soil and, after cultivation, grown for two weeks in a controlled environment cabinet or in a greenhouse, allowed to flower, and mature seeds were harvested and examined by lipid analyses for elongase expression such as Δ6-elongase activity or Δ5- or Δ6-desaturase activity. Lines with increased contents of polyunsaturated C20 and C22 fatty acids were identified in this way.

c) Transformation of Arabidopsis thaliana plants

The protocol of Bechthold et al. (1993) C.R. Acad. Sci. Ser. III Sci. Vie. 316: 1194-1199 was used.

To generate transgenic plants, the generated binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed into Agrobacterium tumefaciens C58C1:pMP90 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788) and, in accordance with the protocol of Bechthold et al. (1993), flowers of Arabidopsis thaliana cv. Columbia 0 were dipped in an agrobacterial solution with OD600=1.0. The procedure was repeated again two days later. Seeds from these flowers were then placed on agar plates with ½ MS, 2% sucrose and 50 mg/l kanamycin. Green seedlings were then transferred to soil.

Example 11: Analysis of plant material of transgenic Orychophragmus or Arabidopsis plants

Extraction of leaf material of transgenic Orychophragmus violaceus and Arabidopsis thaliana plants transformed with pGPTV-D6D5E6(Tp)ω3PiE5D4 and the gas chromatography analysis was carried out as described in example 8. Table 2 shows the results of the analyses. The various fatty acids are indicated in percent by weight. It was possible to show that long-chain polyunsaturated fatty acids were synthesized by both different plant species. It was surprisingly possible with the optimized sequence of the Δ5-elongase (as depicted in SEQ ID NO: 64) from Ostreococcus tauri to obtain a distinctly higher yield of DHA than reported for example by Robert et al. (2005) Functional Plant Biology 32: 473-479 for Arabidopsis thaliana with 1.5% DHA. It was possible for the first time to achieve a synthesis of long-chain polyunsaturated fatty acids for Orychophragmus violaceus.

Example 12: Analysis of seeds of transgenic Brassica juncea lines

Extraction of seeds of transgenic Brassica juncea plants transformed with pSUN-9G, and the gas chromatography analysis was carried out as described in example 8. Table 6 shows the results of the analyses. The various fatty acids are indicated in percent area. As in Wu et al. 2005 it was possible to show the synthesis of long-chain polyunsaturated fatty acids (PUFA).

Surprisingly, the use of the modified elongase sequence OtELO2.2 such as the nucleic acid sequence described by SEQ ID NO: 64 resulted in a drastic increase in the content of C22 fatty acids. In total, the seed oil contained about 8% by weight % polyunsaturated C22 fatty acids. Specifically, the content of the fatty acid docosahexaenoic acid (DHA) in the seed oil was 1.9% by weight %, representing an increase by a factor of 10 compared with Wu et al. 2005.

Example 13: Detailed analysis of the lipid classes and position analysis of leaf material from O. violaceus

About 1 g of leaf tissue was heated in 4 ml of isopropanol at 95°C for 10 minutes, homogenized by Polytron and shaken after addition of 1.5 ml of chloroform. The samples were centrifuged, the supernatant was collected, and the pellet was extracted again with isopropanol:chloroform 1:1 (v/v). The two extracts were combined, dried and dissolved in chloroform. The lipid extract was prefractionated on a silica prepsep column (Fisher Scientific, Nepean, Canada) into neutral lipids, glycolipids and phospholipids, eluting with chloroform:acetic acid 100:1 (v/v), acetone:acetic acid 100:1 (v/v) and methanol:chloroform:water 100:50:40 (v/v/v), respectively. These fractions were further fractionated on silica G-25 thin-layer chromatography plates (TLC; Macherey-Nagel, Düren, Germany). Neutral lipids were developed with hexane:diethyl ether:acetic acid (70:30:1), glycolipids with chloroform:methanol:ammonia (65:25:4 v/v/v) and phospholipids with chloroform:methanol:ammonia:water (70:30:4:1 v/v/v/v). The individual lipid classes were identified after spraying with primulin under UV light, removed by scraping off the plates and either used for direct transmethylation or extracted by a suitable solvent for further analysis.

It was possible by the disclosed methods for the various lipid classes (neutral lipids, phospholipids and galactolipids) to be fractionated and analyzed separately. The glycolipids were additionally examined for the position of the individual fatty acids.

a) Regiospecific analysis of the triacylglycerides (TAG)

Three to five mg of the TLC-purified TAG were dried under nitrogen in a glass tube, resuspended in aqueous buffer by brief ultrasound treatment (1 M Tris pH 8; 2.2% $CaCl_2$ (w/v); 0.05% bile salts (w/v)) and incubated at 40°C for 4 minutes. After addition of 0.1 ml of a solution of pancreatic lipase (10 mg/ml in water), the samples were vigorously vortexed for 3 minutes, and the digestion was stopped by adding 1 ml of ethanol and 1.5 ml of 4 M

HCl. The partly digested TAGs were extracted twice with diethyl ether, washed with water, dried and dissolved in a small volume of chloroform. Monoacylglycerols (MAG) were separated from the free fatty acids and undigested TAGs on a TLC plate as described above for neutral lipids. The point corresponding to the MAGs was analyzed by GC and represented the sn-2 position of the TAGs. The distribution of the fatty acids to the remaining sn-1 and sn-3 positions was calculated by the following formula: sn-1 + sn-3 = (TAG × 3 - MAG)/2.

This position analysis of the triacylglycerides revealed in this case that EPA and DHA are present in similar concentrations in the sn-2 and sn-1/3 positions, while ARA is to be found overall only in small amounts in the triacylglycerides, and here mainly in the sn-2 position (Tab. 3).

b) Stereospecific analysis of phospholipids

Fractionated and extracted phosphatidylglycol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were dried under N_2 and resuspended in 0.5 ml of borate buffer (0.5M, pH 7.5, containing 0.4 mM $CaCl_2$). After a brief ultrasound treatment, 5U of phospholipase A2 from the venom of Naja mossambica (Sigma P-7778) and 2 ml of diethyl ether were added and the samples were vortexed at room temperature for 2 hours. The ether phase was dried, the digestion was stopped with 0.3 ml of 1M HCl, and the reaction mixture was extracted with chloroform:methanol (2:1 v/v). The digested phospholipids were separated by TLC in chloroform:methanol:ammonia:water (70:30:4:2 v/v/v/v) and points which corresponded to the liberated free fatty acids and lysophospholipids were removed by scraping and directly transmethylated.

Positional analysis of the phospholipids showed an accumulation of EPA and DHA in the sn-2 position of phosphatidylcholine (PC), while DHA was similarly distributed in sn-1 and sn-2 position in phosphatidylethanolamine (PE). Only traces of, or no, ARA was to be found in both phospholipids (Tab. 4). The concentrations of EPA and DHA in phosphatidylglycerol were lower than in the other investigated phospholipids, with accumulation in the sn-2 position also to be observed in this lipid class (Tab. 4, PG).

c) Stereospecific analysis of glycolipids

The galactolipids were investigated as a further polar lipid class. Galactolipids are found in the membranes of plastids and form the main components there.

TLC-purified monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol

(DGDG) were dried under nitrogen and dissolved in 0.5 ml of diethyl ether. Then 25 units of the lipase from Rhizopus arrhizus (Sigma 62305), resuspended in 2 ml of borate buffer (50 mM, pH 7.5 containing 2 mM $CaCl_2$), were added, and the samples were vortexed at room temperature for 2 hours. The ether phase was dried and the digestion was stopped by adding 0.3 ml of 1M HCl, and the lipids were extracted with 4 ml of chloroform:methanol (2:1 v/v). After drying, the digested galactolipids were in a small volume of chloroform:methanol (2:1 v/v) and developed twice on a precoated silica TLC plate, firstly with chloroform:methanol: ammonia:water (70:30:4:1 v/v/v/v) to about two thirds the height of the plate, followed by complete development in hexane:diethyl ether:acetic acid (70:30:1). The points which corresponded to the liberated free fatty acids and the lysogalactolipids were identified after spraying with primulin, scraped off and transmethylated directly for GC analysis.

It was possible to find VLCPUFA in these lipids too, with an accumulation of EPA in the sn-2 position being observed. DHA was to be found only in the digalactodiacylglycerols (DGDG) and was undetectable in the monogalactodiacylglycerols (MGDG) (Table 5). The distribution of VLCPUFA in galactolipids, a compartment in which these fatty acids were not expected, shows the dynamics of the synthesis and the later transformation. VLCPUFA in polar lipids are of particular nutritional value because they can be absorbed better in the intestines of mammals than the neutral lipids.

Table 1: Test of the optimized sequences of pOTE1.1 and pOTE2.1 in yeast. The conversion rates were determined in accordance with the substrate conversions. A distinct rise in activity was achievable with the optimized sequence in plasmid pOTE2.2.

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Conversion rates of the Ostreococcus tauri elongases					
	Genes	**Substrate Product**	**GLA 20:3**	**ARA 22:4**	**EPA 22:5**
pOTE1.1	**d6Elongase(Ot)**		**21.1**		
pOTE1.2	**d6Elongase(Ot)_opt**		**25.6**		
pOTE2.1	**d5Elongase(Ot)**			**7.3**	**35.9**
pOTE2.2	**d5Elongase(Ot)_opt**			**32.7**	**63.1**

Table 2: Gas chromatographic analysis of leaf material of Orychophragmus violaceus and Arabidopsis thaliana. The individual fatty acids are indicated in percent area.

Fatty acid composition of leaf material of Orychophragmus violaceus											
Fatty acids	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Control	20.9	8.5	3.3	16.0	0.0	47.4	0.0	0.0	0.0	0.0	0.0
Transgene	21.3	8.2	5.2	5.2	4.2	23.1	5.0	0.6	13.5	2.7	4.5

Fatty acid composition of leaf material of Arabidopsis thaliana											
Fatty acids	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Control	12.8	10.0	3.5	14.2	0.0	54.6	0.0	0.0	0.0	0.0	0.0
Transgene	19.3	8.5	5.0	4.6	6.4	31.0	4.4	0.0	6.3	1.5	6.3

Table 3: Regiospecific analysis of the triacylglycerides from leaf material from transgenic O. violaceus plants.

TAG	16:0	18:0	18:1n-9	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
wt	25,12	3,03	5,06		18,53		44,72							
sn-2	1,42	0,76	6,79		27,62		62,03							
sn-1+3	36,97	4,17	4,19		13,98		36,07							
Transgene	22,63	3,12	3,46	0,77	2,36	9,51	6,37	13,03	0,74	0,83	3,87	24,96	2,22	4,15
sn-2	1,62	0,64	8,33	1,61	5,15	16,21	10,88	19,84	0,17	1,38	1,99	24,82	3,27	3,02
sn-1+3	33,13	4,39	1,02	0,35	0,96	6,16	4,11	9,63	1,02	0,55	4,59	25,03	1,59	4,72

Table 4: Stereospecific analysis of the phospholipids from leaf material from transgenic O. violaceus plants.

PG	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**27,96**	**20,04**	**4,11**	**2,89**	**0,90**		**21,82**	**0,00**	**21,56**							
sn-1	17,26	0,53	2,61	3,82	1,91		39,01	0,00	34,44							
sn-2	38,66	39,56	5,62	1,96	0,00		4,62	0,00	8,69							
Transgene	**27,15**	**24,70**	**3,08**	**4,62**	**1,20**	**0,00**	**15,15**	**1,53**	**17,94**	**1,40**	**0,00**	**0,00**	**0,45**	**2,18**	**0,10**	**0,58**
sn-1	21,16	3,61	4,23	7,52	2,14		27,40	0,50	31,57	0,81			0,38	1,24	0,00	0,33
sn-2	33,15	45,79	1,94	1,71	0,27		2,90	2,57	4,30	2,00			0,51	3,13	0,27	0,83

PE	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**37,49**	**0,00**	**6,62**	**4,35**	**1,37**		**19,28**		**29,95**							
sn-1	54,22	0,00	7,74	3,39	3,42		12,64		13,71							
sn-2	20,77	0,00	5,51	5,31	0,00		25,93		46,18							
Transgene	**31,78**	**0,81**	**5,84**	**3,08**	**2,20**	**0,85**	**5,57**	**11,25**	**11,34**	**7,38**	**0,00**	**0,00**	**2,88**	**9,41**	**1,90**	**4,90**
sn-1	50,17	0,33	10,86	3,22	4,94	0,35	2,63	3,27	3,59	2,31	0,56		4,42	6,18	0,38	4,19
sn-2	13,40	1,29	0,83	2,95	0,00	1,35	8,50	19,23	19,10	12,45	0,00		1,34	12,64	3,41	5,61

PC	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**27,67**	**0,84**	**6,38**	**8,56**	**1,80**		**21,75**		**33,01**							
sn-1	48,05	0,44	8,65	5,05	3,41		14,52		18,04							
sn-2	7,28	1,24	4,11	12,06	0,18		28,97		47,98							
Transgene	**21,00**	**0,00**	**8,01**	**10,02**	**2,86**	**1,25**	**3,77**	**11,63**	**5,60**	**12,11**	**0,50**	**0,00**	**4,34**	**11,16**	**3,76**	**3,70**
sn-1	45,35	0,00	14,71	5,08	5,70	0,31	3,23	3,09	4,53	2,65	0,61	0,08	4,01	8,32	0,41	1,18
sn-2	3,36	0,00	1,30	14,96	0,02	2,20	4,31	20,18	6,62	21,56	0,38	0,00	4,66	13,99	7,12	6,22

Table 5: Stereospecific analysis of the galactolipids from leaf material from transgenic O. violaceus plants.

MGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**2.64**	**0.13**	**1.23**	**30.72**	**0.33**	**0.35**	**0.26**		**3.81**		**60.52**					
sn-1	0.00	0.05	0.00	7.11	0.35	0.31	0.41		4.60		87.30					
sn-2	5.34	0.21	2.55	54.34	0.31	0.39	0.12		3.01		33.74					
tr	**4.16**	**0.20**	**1.08**	**33.81**	**0.93**	**0.73**	**0.52**	**0.03**	**1.64**	**1.88**	**44.82**	**2.73**	**0.04**	**0.30**	**0.50**	**5.08**
sn-1	1.22	0.29	0.54	4.79	1.51	1.15	0.93	0.00	2.80	0.14	80.19	0.00	0.08	0.17	0.87	3.86
sn-2	7.11	0.11	1.61	62.82	0.34	0.31	0.11	0.11	0.47	3.62	9.46	5.48	0.00	0.43	0.14	6.31

DGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**17.67**	**0.19**	**0.38**	**2.15**	**1.61**	**0.51**	**0.94**		**5.55**		**70.71**					
sn-1	16.84	0.25	0.50	2.52	2.21	0.55	1.75		6.07	0.00	68.74					
sn-2	18.50	0.12	0.27	1.78	1.01	0.46	0.13		5.05		72.68					
tr	**18.50**	**0.00**	**0.00**	**2.62**	**2.84**	**1.36**	**1.39**	**0.00**	**6.28**	**3.55**	**54.66**	**0.00**	**0.00**	**0.00**	**2.18**	**5.36**
sn-1	22.74	0.17	0.23	0.48	4.55	1.71	2.32	0.24	9.22	0.23	55.06	0.27	0.00	0.00	0.36	1.23
sn-2	14.27	0.00	0.00	4.77	1.12	1.00	0.46	0.00	3.33	6.88	53.26	0.00	0.00	0.00	4.01	9.49

Table 6: Gas chromatographic determination of the fatty acids from seeds of transgenic Brassica juncea plants transformed with the construct pSUN-9G in percent by weight. WT describes the unmodified wild-type control.

	Lipid Profile(%)							
	16:0	18:0	18:1	18:2	γ18:3	α18:3	18:4	20:0
BJ223_PUFA184_MKP71_581A	4,4	3,0	22,5	16,9	27,0	4,9	3,2	0,6
BJ223_PUFA184_MKP71_581A	4,7	3,9	17,9	10,6	29,5	4,2	4,0	0,9
BJ223_PUFA184_MKP71_581A	4,4	3,0	18,9	13,8	30,5	4,1	3,2	0,7
BJ223_PUFA184_MKP71_581A	4,6	3,3	20,5	13,2	29,8	4,2	3,3	0,8

20:3 (8,11,14)	20:3 (11,14,17)	20:4 (ARA) (5,8,11,14)	20:4 (ETeA) (8,11,14,17)	20:5 (EPA) (5,8,11,14,17)	22:1	22:4	22:5	22:6
1,1	0,5	3,1	0,6	4,6	0,0	1,5	2,0	1,5
2,0	0,9	4,2	1,0	4,9	0,0	3,1	3,5	1,9
1,3	0,7	4,1	0,5	4,5	0,0	2,7	2,6	1,6
1,4	0,6	3,6	0,6	4,4	0,0	2,4	2,5	1,6

We Claim:

1. Oils, lipids and/or fatty acids produced by a transgenic Brassica plant, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant, wherein said polyunsaturated fatty acids comprise at least 20% by weight of eicosapentaenoic acid (EPA), at least 2% by weight of docosapentaenoic acid (DPA), and/or at least 4% by weight of docosahexaenoic acid (DHA) based on the total fatty acids in the transgenic plant in form of triacylglycerides.

2. The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-1, sn-2 or sn-3 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 1.5% by weight of DPA is present in the sn-1, sn-2 or sn-3 position; and/or

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-1, sn-2 or sn-3 position.

3. The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-2 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 3% by weight of DPA is present in the sn-2 position; and/or

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-2 position.

4. The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise:

a) at least 20% by weight of EPA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides;

b) at least 20% by weight of EPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides; or

c) at least 2% by weight of DPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

5. The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

6. The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

7. The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

8. The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

9. Oils, lipids and/or fatty acids produced by a transgenic Brassica plant, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

10. The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant.

11. The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 20% by weight of EPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

12. The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

13. The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

14. The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total

fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

15. The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

16. The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

17. A method for producing oils, lipids and/or fatty acids of claim 1, comprising expressing in a Brassica plant a nucleic acid encoding a Δ6-desaturase, a nucleic acid encoding a Δ5-desaturase, a nucleic acid encoding a Δ6-elongase, a nucleic acid encoding a ω3-desaturase, a nucleic acid encoding a Δ5-elongase, and a nucleic acid encoding a Δ4-desaturase, wherein said nucleic acid encoding a Δ5-elongase is codon-optimized by adapting to the codon usage of Brassica.

18. The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a nucleotide sequence having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 64, and wherein said nucleotide sequence is obtained by adapting at least 30% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of Brassica.

19. The method of claim 18, wherein said nucleotide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 64, or wherein said nucleotide sequence encodes a polypeptide having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65.

20. The method of claim 18, wherein said nucleotide sequence is adapted taking into account the natural frequency of individual codons.

21. The method of claim 18, wherein said nucleotide sequence is obtained by adapting at least 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of Brassica.

22. The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a translated section coding for a protein having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65, and wherein the translated

section comprises a nucleotide sequence obtained by adapting at least 30% or 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of Brassica.

Abstract

The invention relates to a method for producing eicosapentanoic acid, docosapentanoic acid and/or docohexanoic acid in transgenic plants. According to said method, the plant is provided with at least one nucleic acid sequence coding for a polypetide with a Δ6 desaturase activity, at least one nucleic acid sequence coding for a polypeptide with a Δ6 elongase activity, at least one nucleic acid sequence coding for a polypeptide with a Δ5 desaturase activity, and at least one nucleic acid sequence coding for a polypeptide with a Δ5 elongase activity, the nucleic acid sequence coding for a polypeptide with a Δ5 elongase activity being modified in relation to the nucleic acid sequence in the organism from which the sequence originates, such that it is adapted to the codon use in at least one type of plant. For the production of docosahexanoic acid, at least one nucleic acid sequence coding for a polypeptide with a Δ4 desaturase activity is also introduced into the plant.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Filer:	Hui-Ju Wu/Jamie Jensen-Smith
Attorney Docket Number:	074017-0013-01-US-541474

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Claims:				
Claims in Excess of 20	1202	2	80	160
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1760**

Electronic Acknowledgement Receipt

EFS ID:	26838159
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Hui-Ju Wu/Jamie Jensen-Smith
Filer Authorized By:	Hui-Ju Wu
Attorney Docket Number:	074017-0013-01-US-541474
Receipt Date:	06-SEP-2016
Filing Date:	
Time Stamp:	12:59:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1760
RAM confirmation Number	090616INTEFSW13005900
Deposit Account	1053
Authorized User	Jamie Jensen-Smith

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	Transmittal.pdf	18795 2c13f10e97d3b6562bbeeb1a9aa87aa3996fe8d5	no	1
Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	24984 a1e2a57a3ee9f6d1e52615a43cb5c007b4fe922b	no	7
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
3	Oath or Declaration filed	Declaration.pdf	141789 6c7ce1f0e7554307ac8a0ef8dcecf9ad59d60436	no	4
Warnings:					
Information:					
4	Power of Attorney	POA.pdf	106588 8bd275611c01890b3110785fea4a15280dc36544	no	1
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
5	Transmittal Letter	InformationDisclosureStatement.pdf	15340 2d937b43ade2f41d49a0e9c54ebbcd6d7218fe28	no	2
Warnings:					
Information:					
6	Information Disclosure Statement (IDS) Form (SB08)	SB08.pdf	80562 9a331c7998bce5e445eb53198aa4df97c8a4f9d3	no	6

Warnings:					
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This is not an USPTO supplied IDS fillable form					
7		Specification.pdf	767821 a92f2f0841d9558ad609cfbd59702c3b746c46bb	yes	90
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	85	
	Claims		86	89	
	Abstract		90	90	
Warnings:					
Information:					
8	Sequence Listing (Text File)	Sequence_Listing_074017_0013_01.txt	747403	no	-
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	36905 d191b72e3f69ac11134a85b0d700f0fcbe6dcaea	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1940187		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 15256914 Document Date: 09/06/2016

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

Electronic Acknowledgement Receipt

EFS ID:	26838159
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
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Receipt Date:	06-SEP-2016
Filing Date:	
Time Stamp:	12:59:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
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Payment was successfully received in RAM	$1760
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Deposit Account	500573
Authorized User	Jamie Jensen-Smith

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37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	Transmittal.pdf	18795 2c13f10e97d3b6562bbeeb1a9aa87aa3996fe8d5	no	1
Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	24984 a1e2a57a3ee9f6d1e52615a43cb5c007b4fe922b	no	7
Warnings:					
This is not an USPTO supplied ADS fillable form					
Information:					
3	Oath or Declaration filed	Declaration.pdf	141789 6c7ce1f0e7554307ac8a0ef8dcecf9ad59d60436	no	4
Warnings:					
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4	Power of Attorney	POA.pdf	106588 8bd275611c01890b3110785fea4a15280dc36544	no	1
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
5	Transmittal Letter	InformationDisclosureStatement.pdf	15340 2d937b43ade2f41d49a0e9c54ebbcd6d7218fe28	no	2
Warnings:					
Information:					
6	Information Disclosure Statement (IDS) Form (SB08)	SB08.pdf	80562 9a331c7998bce5e445eb53198aa4df97c8a4f9d3	no	6

Warnings:					
This is not an USPTO supplied IDS fillable form					
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7		Specification.pdf	767821 a92f2f0841d9558ad609cfbd59702c3b746c46bb	yes	90
	Multipart Description/PDF files in .zip description				
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8	Sequence Listing (Text File)	Sequence_Listing_074017_0013_01.txt	747403	no	-
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	36905 d191b72e3f69ac11134a85b0d700f0fcbe6dcaea	no	2
Warnings:					
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		Total Files Size (in bytes):	1940187		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Douglas, Keisha (ASRC)
Timestamp: [year=2016; month=9; day=9; hr=16; min=33; sec=49; ms=209; ]
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Validated By CRFValidator v 1.0.5

Application No: 15256914 Version No: 1.0

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Output Set:

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Finished: 2016-09-06 13:01:39.665
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Total Errors: 0
No. of SeqIDs Defined: 221
Actual SeqID Count: 221

Error code	Error Description
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W 402	Undefined organism found in <213> in SEQ ID (2)
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W 402	Undefined organism found in <213> in SEQ ID (8)
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W 213	Artificial or Unknown found in <213> in SEQ ID (43)
W 447	n or Xaa used, for: SEQID(43) on line number 3922
W 447	n or Xaa used, for: SEQID(43) on line number 3924
W 447	n or Xaa used, for: SEQID(43) on line number 3926
W 447	n or Xaa used, for: SEQID(43) on line number 3928
W 447	n or Xaa used, for: SEQID(43) on line number 3930
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W 447	n or Xaa used, for: SEQID(43) on line number 3934
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Input Set:

Output Set:

Started: 2016-09-06 13:01:04.541
Finished: 2016-09-06 13:01:39.665
Elapsed: 0 hr(s) 0 min(s) 35 sec(s) 124 ms
Total Warnings: 470
Total Errors: 0
No. of SeqIDs Defined: 221
Actual SeqID Count: 221

Error code	Error Description
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W 447	n or Xaa used, for: SEQID(43) on line number 3946
W 447	n or Xaa used, for: SEQID(43) on line number 3948
W 447	n or Xaa used, for: SEQID(43) on line number 3950
W 447	n or Xaa used, for: SEQID(43) on line number 3952
W 447	n or Xaa used, for: SEQID(43) on line number 3954
W 447	n or Xaa used, for: SEQID(43) on line number 3956
W 447	n or Xaa used, for: SEQID(43) on line number 3958
W 447	n or Xaa used, for: SEQID(43) on line number 3960
	This error has occured more than 20 times, will not be displayed
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Input Set:

Output Set:

Started: 2016-09-06 13:01:04.541
Finished: 2016-09-06 13:01:39.665
Elapsed: 0 hr(s) 0 min(s) 35 sec(s) 124 ms
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SEQUENCE LISTING

<110> Cirpus, Petra
Bauer, Jorg
Qiu, Xiao
Wu, Guohai
Cheng, Bifang
Truksa, Martin
Wetjen, Tom

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Ile Arg Glu His Ala Thr Pro Ala Thr Ala Trp Ile Val Ile His His
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35 40 45
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gag gag cgc gcg cgc cgc gag cgc atc aac gag ttc atc gcg tcc tac       336
Glu Glu Arg Ala Arg Arg Glu Arg Ile Asn Glu Phe Ile Ala Ser Tyr
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        35                  40                  45
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His Pro Ser Ser Ala Leu Lys Leu Leu Glu Gln Phe Tyr Val Gly Asp
65                  70                  75                  80
Val Asp Glu Thr Ser Lys Ala Glu Ile Glu Gly Glu Pro Ala Ser Asp
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Glu Glu Arg Ala Arg Arg Glu Arg Ile Asn Glu Phe Ile Ala Ser Tyr
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Arg Arg Leu Arg Val Lys Val Lys Gly Met Gly Leu Tyr Asp Ala Ser
        115                 120                 125
Ala Leu Tyr Tyr Ala Trp Lys Leu Val Ser Thr Phe Gly Ile Ala Val
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385                 390                 395                 400
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                405                 410                 415
Leu Ile Lys Ser Leu Cys Lys Glu Phe Asp Ile Pro Phe His Glu Thr
            420                 425                 430
Gly Phe Trp Glu Gly Ile Tyr Glu Val Val Asp His Leu Ala Asp Ile
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Ser Lys Glu Phe Ile Thr Glu Phe Pro Ala Met
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1               5                   10                  15
tcg atc agt caa cgt att cat gat gga gat gaa gag gct atg aag ttc       96
Ser Ile Ser Gln Arg Ile His Asp Gly Asp Glu Glu Ala Met Lys Phe
            20                  25                  30
att att atc gac aag aag gtg tac gat gtt act gaa ttt att gaa gac      144
Ile Ile Ile Asp Lys Lys Val Tyr Asp Val Thr Glu Phe Ile Glu Asp
        35                  40                  45
cac cct ggt ggt gct caa gtt ttg ttg act cat gtt ggg aaa gat gca      192
His Pro Gly Gly Ala Gln Val Leu Leu Thr His Val Gly Lys Asp Ala
    50                  55                  60
tct gac gta ttt cat gcc atg cat ccc gaa tct gct tac gaa gtc ttg      240
Ser Asp Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Val Leu
65                  70                  75                  80
aac aat tac ttc gta ggc gat gtt caa gaa aca gta gtc act gaa aaa      288
Asn Asn Tyr Phe Val Gly Asp Val Gln Glu Thr Val Val Thr Glu Lys
                85                  90                  95
tct tct tct gcc caa ttc gct gta gaa atg cgt cag ctt cgg gat caa      336
Ser Ser Ser Ala Gln Phe Ala Val Glu Met Arg Gln Leu Arg Asp Gln
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ttg aaa aag gaa ggt tat ttc cac tcc agc aaa ctc ttt tat gct tac      384
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Leu Lys Lys Glu Gly Tyr Phe His Ser Ser Lys Leu Phe Tyr Ala Tyr
        115                 120                 125
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Lys Val Leu Ser Thr Leu Ala Ile Cys Ile Ala Gly Leu Ser Pro Leu
    130                 135                 140
tat gct tat ggt cgt act tcc act ttg gct gtc gtc gca tct gct att       480
Tyr Ala Tyr Gly Arg Thr Ser Thr Leu Ala Val Val Ala Ser Ala Ile
145                 150                 155                 160
act gtt ggt atc ttt tgg caa cag tgt ggt tgg ctg gct cac gat ttc       528
Thr Val Gly Ile Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe
                165                 170                 175
gga cat cat caa tgc ttc gaa gac cgc act tgg aac gat gtt ctc gtc       576
Gly His His Gln Cys Phe Glu Asp Arg Thr Trp Asn Asp Val Leu Val
            180                 185                 190
gta ttt ctc gga aac ttt tgt caa gga ttt tca tta tca tgg tgg aag       624
Val Phe Leu Gly Asn Phe Cys Gln Gly Phe Ser Leu Ser Trp Trp Lys
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aac aag cac aac aca cat cac gca agt aca aat gtt cat gga cag gat       672
Asn Lys His Asn Thr His His Ala Ser Thr Asn Val His Gly Gln Asp
    210                 215                 220
ccc gac att gac aca gcc cct gtt ttg ttg tgg gat gaa tat gct tct       720
Pro Asp Ile Asp Thr Ala Pro Val Leu Leu Trp Asp Glu Tyr Ala Ser
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Phe Leu Ala Glu Gln Val Leu Pro His Gln Thr Arg Tyr Phe Phe Phe
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Tyr Ser Trp Cys Ser Asn Val Tyr His Met Val Leu Phe Phe Leu Val
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385                 390                 395                 400
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        435                 440                 445
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            20                  25                  30
Ile Ile Ile Asp Lys Lys Val Tyr Asp Val Thr Glu Phe Ile Glu Asp
        35                  40                  45
His Pro Gly Gly Ala Gln Val Leu Leu Thr His Val Gly Lys Asp Ala
    50                  55                  60
Ser Asp Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Val Leu
65                  70                  75                  80
Asn Asn Tyr Phe Val Gly Asp Val Gln Glu Thr Val Val Thr Glu Lys
                85                  90                  95
Ser Ser Ser Ala Gln Phe Ala Val Glu Met Arg Gln Leu Arg Asp Gln
            100                 105                 110
Leu Lys Lys Glu Gly Tyr Phe His Ser Ser Lys Leu Phe Tyr Ala Tyr
        115                 120                 125
Lys Val Leu Ser Thr Leu Ala Ile Cys Ile Ala Gly Leu Ser Pro Leu
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Thr Val Gly Ile Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe
                165                 170                 175
Gly His His Gln Cys Phe Glu Asp Arg Thr Trp Asn Asp Va
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/256,914	09/06/2016	1629	1760	074017-0013-01-US	22	2

CONFIRMATION NO. 4050

123223
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

FILING RECEIPT

OC000000085751731

Date Mailed: 09/14/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

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Applicant(s)

BASF Plant Science GmbH, Ludwigshafen, GERMANY;

Power of Attorney: The patent practitioners associated with Customer Number 123223

Domestic Priority data as claimed by applicant

This application is a CON of 12/280,090 08/20/2008
which is a 371 of PCT/EP2007/051675 02/21/2007

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 06120309.7 09/07/2006
GERMANY 102006008030.0 02/21/2006

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 09/13/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/256,914**

Projected Publication Date: 12/22/2016

Non-Publication Request: No

Early Publication Request: No

Title

METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/256,914

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	22 minus 20 =	* 2			OR	x 80 =	160
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1760

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US

CONFIRMATION NO. 4050

123223
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

INFORMAL NOTICE

OC000000085751732

Date Mailed: 09/14/2016

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

A new inventor's oath or declaration that identifies this application (e.g., by Application Number and filing date) is required. The inventor's oath or declaration does not comply with 37 CFR 1.63 in that it:

- does not state that the above-identified application was made or authorized to be made by the person executing the oath or declaration.

Petra Cirpus
Jörg Bauer
Xiao Qiu
Guohai Wu
Bifang Cheng
Martin Truksa
Tom Wetjen

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/kgebremichael/

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GD1

European Patent Office
DG1

Office européen des brevets
DG1

Bescheinigung

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

Certificate

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Attestation

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécificée à la page suivante.

Patentanmeldung Nr. **Patent application No.** **Demande de brevet n°**

06120309.7 / EP06120309

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP06120309

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

R.C. van Dijk

Anmeldung Nr:
Application no.: 06120309.7
Demande no:

Anmeldetag:
Date of filing: 07.09.06
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

BASF Plant Science GmbH
Carl-Bosch-Strasse 64
67117 Limburgerhof/DE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren

In anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation / International Patent Classification / Classification internationale de brevets:

C12N1/00

Am Anmeldetag benannte Vertragstaaten / Contracting states designated at date of filing / Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR

Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren

Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung von Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in transgenen Pflanzen, indem in der Pflanze bereitgestellt werden mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Desaturase-Aktivität kodiert; und mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert,
wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist.

In einer bevorzugten Ausführungsform werden zusätzlich weitere Nukleinsäuresequenzen, die für ein Polypeptid mit der Aktivität einer ω3-Desaturase und/oder einer Δ4-Desaturase kodieren, in der Pflanze bereitgestellt.

In einer weiteren bevorzugten Ausführungsform werden weitere Nukleinsäuresequenzen, die für Acyl-CoA-Dehydrogenase(n), Acyl-ACP(= acyl carrier protein)–Desaturase(n), Acyl–ACP–Thioesterase(n), Fettsäure–Acyl–Transferase(n), Acyl-CoA:Lysophospholipid-Acyltransferase(n), Fettsäure–Synthase(n), Fettsäure–Hydroxylase(n), Acetyl-Coenzym A–Carboxylase(n), Acyl–Coenzym A–Oxidase(n), Fettsäure–Desaturase(n), Fettsäure–Acetylenasen, Lipoxygenasen, Triacylglycerol–Lipasen, Allenoxid–Synthasen, Hydroperoxid–Lyasen oder Fettsäure–Elongase(n) kodieren, in der Pflanze bereitgestellt.

Die Erfindung betrifft weiterhin rekombinante Nukleinsäuremoleküle, umfassend mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ5-

Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert; und mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert und die gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist.

Ein weiterer Teil der Erfindung betrifft Öle, Lipide und/oder Fettsäuren, die nach dem erfindungsgemäßen Verfahren hergestellt wurden, und deren Verwendung.

Schließlich betrifft die Erfindung auch transgene Pflanzen, die nach dem erfindungsgemäßen Verfahren hergestellt wurden oder die ein erfindungsgemäßes rekombinantes Nukleinsäuremolekül enthalten, und deren Verwendung als Nahrungs- oder Futtermittel.

Die Lipidsynthese lässt sich in zwei Abschnitte unterteilen: die Synthese von Fettsäuren und ihre Bindung an sn-Glycerin-3-Phosphat sowie die Addition oder Modifikation einer polaren Kopfgruppe. Übliche Lipide, die in Membranen verwendet werden, umfassen Phospholipide, Glycolipide, Sphingolipide und Phosphoglyceride. Die Fettsäuresynthese beginnt mit der Umwandlung von Acetyl-CoA in Malonyl-CoA durch die Acetyl-CoA-Carboxylase oder in Acetyl-ACP durch die Acetyltransacylase. Nach einer Kondensationsreaktion bilden diese beiden Produktmoleküle zusammen Acetoacetyl-ACP, das über eine Reihe von Kondensations-, Reduktions- und Dehydratisierungsreaktionen umgewandelt wird, so dass ein gesättigtes Fettsäuremolekül mit der gewünschten Kettenlänge erhalten wird. Die Produktion der ungesättigten Fettsäuren aus diesen Molekülen wird durch spezifische Desaturasen katalysiert, und zwar entweder aerob mittels molekularem Sauerstoff oder anaerob (bezüglich der Fettsäuresynthese in Mikroorganismen siehe F.C. Neidhardt et al. (1996) E. coli und Salmonella. ASM Press: Washington, D.C., S. 612-636 und darin enthaltene Literaturstellen; Lengeler et al. (Hrsgb.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, und die enthaltene Literaturstellen, sowie Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 und die enthaltenen Literaturstellen). Die so hergestellten an Phospholipide gebundenen Fettsäuren müssen anschließend für die weiteren Elongationen aus den Phospholipiden wieder in den Fett-

säureCoA-Ester-Pool überführt werden. Dies ermöglichen Acyl-CoA:Lysophospholipid-Acyltransferasen. Weiterhin können diese Enzyme die elongierten Fettsäuren wieder von den CoA-Estern auf die Phospholipide übertragen. Diese Reaktionsabfolge kann gegebenenfalls mehrfach durchlaufen werden.

Ferner müssen Fettsäuren anschließend an verschiedene Modifikationsorte transportiert und in das Triacylglycerin-Speicherlipid eingebaut werden. Ein weiterer wichtiger Schritt bei der Lipidsynthese ist der Transfer von Fettsäuren auf die polaren Kopfgruppen, beispielsweise durch Glycerin-Fettsäure-Acyltransferase (siehe Frentzen, 1998, Lipid, 100(4-5):161-166).

Eine Übersicht über die Pflanzen-Fettsäurebiosynthese, Desaturierung, den Lipidstoffwechsel und Membrantransport von fetthaltigen Verbindungen, die Betaoxidation, Fettsäuremodifikation und Cofaktoren, Triacylglycerin-Speicherung und –Assemblierung geben einschließlich der Literaturstellen die folgenden Artikel: Kinney (1997) Genetic Engeneering, Hrsgb.: JK Setlow, 19:149-166; Ohlrogge und Browse (1995) Plant Cell 7:957-970; Shanklin und Cahoon (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker (1996) Genetic Engeneering, Hrsgb.: JK Setlow, 18:111-13; Gerhardt (1992) Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl (1995) Biochim. Biophys Acta 1256:181-186; Kunau et al. (1995) Prog. Lipid Res. 34:267-342; Stymne et al. (1993) in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Hrsgb.: Murata und Somerville, Rockville, American Society of Plant Physiologists, 150-158; Murphy & Ross (1998) Plant Journal. 13(1):1-16.

Die mehrfach ungesättigten Fettsäuren können entsprechend ihrem Desaturierungsmuster in zwei große Klassen, die ω-6- und die ω-3-Fettsäuren, eingeteilt werden, die metabolisch und funktionell unterschiedliche Aktivitäten haben.

Im Folgenden werden mehrfach ungesättigte Fettsäuren als PUFA, PUFAs, LCPUFA oder LCPUFAs bezeichnet (poly unsaturated fatty acids, PUFA, mehrfach ungesättigte Fettsäuren; long chain poly unsaturated fatty acids, LCPUFA, langkettige mehrfach ungesättigte Fettsäuren).

Als Ausgangsstoff für den ω-6-Stoffwechselweg fungiert die Fettsäure Linolsäure (18:2$^{\Delta 9,12}$), während der ω-3-Weg über Linolensäure (18:3$^{\Delta 9,12,15}$) abläuft. Linolensäure wird dabei durch die Aktivität einer ω-3-Desaturase aus Linolsäure gebildet (Tocher et al. (1998) Prog. Lipid Res. 37: 73-117 ; Domergue et al. (2002) Eur. J. Biochem. 269: 4105-4113).

Säugetiere und damit auch der Mensch verfügen über keine entsprechende Desaturase-aktivität (Δ-12- und ω-3-Desaturase) zur Bildung dieser Ausgangsstoffe und müssen daher diese Fettsäuren (essentielle Fettsäuren) über die Nahrung aufnehmen. Über eine Abfolge von Desaturase- und Elongase-Reaktionen werden dann aus diesen Vorstufen die physiologisch wichtigen mehrfach ungesättigten Fettsäuren Arachidonsäure (= ARA, 20:4$^{\Delta 5,8,11,14}$), eine ω-6-Fettsäure und die beiden ω-3-Fettsäuren Eicosapentaen- (= EPA, 20:5$^{\Delta 5,8,11,14,17}$) und Docosahexaensäure (DHA, 22:6$^{\Delta 4,7,10,13,17,19}$) synthetisiert.

Die Verlängerung von Fettsäuren durch Elongasen um 2 bzw. 4 C-Atome ist für die Produktion von C_{20}- bzw. C_{22}-PUFAs von entscheidender Bedeutung. Dieser Prozess verläuft über 4 Stufen. Den ersten Schritt stellt die Kondensation von Malonyl-CoA an das Fettsäure-Acyl-CoA durch die Ketoacyl-CoA-Synthase (KCS, im weiteren Text als Elongase bezeichnet) dar. Es folgt dann ein Reduktionsschritt (Ketoacyl-CoA-Reduktase, KCR), ein Dehydratationsschritt (Dehydratase) und ein abschließender Reduktionsschritt (Enoyl-CoA-Reduktase). Es wurde postuliert, dass die Aktivität der Elongase die Spezifität und Geschwindigkeit des gesamten Prozesses beeinflusst (Millar and Kunst (1997) Plant Journal 12:121-131).

Für Fettsäuren und Triacylglyceride besteht eine Vielzahl von Anwendungen in der Lebensmittelindustrie, der Tierernährung, der Kosmetik und im Pharmabereich.
Je nachdem, ob es sich um freie gesättigte und ungesättigte Fettsäuren oder um Triacylglyceride mit einem erhöhten Gehalt an gesättigten oder ungesättigten Fettsäuren handelt, sind sie für die unterschiedlichsten Anwendungen geeignet. So werden z.B. in der humanen Ernährung Lipide mit ungesättigten, speziell mehrfach ungesättigten, Fettsäuren bevorzugt. Den mehrfach ungesättigten ω-3-Fettsäuren wird dabei ein positiver

Effekt auf den Cholesterinspiegel im Blut und damit auf die Prävention einer Herzerkrankung zugeschrieben. Durch Zugabe dieser ω-3-Fettsäuren zur Nahrung kann das Risiko einer Herzerkrankung, eines Schlaganfalls oder von Bluthochdruck deutlich verringert werden (Shimikawa (2001) World Rev. Nutr. Diet. 88: 100-108).

Auch entzündliche, speziell chronisch entzündliche, Prozesse im Rahmen immunologischer Erkrankungen wie rheumatoider Arthritis lassen sich durch ω-3-Fettsäuren positiv beeinflussen (Calder (2002) Proc. Nutr. Soc. 61: 345-358; Cleland und James (2000) J. Rheumatol. 27: 2305-2307). Sie werden deshalb Lebensmitteln, speziell diätetischen Lebensmitteln, zugegeben oder finden in Medikamenten Anwendung. ω-6-Fettsäuren wie Arachidonsäure üben bei diesen rheumatischen Erkrankungen eher einen negativen Effekt aus.

ω-3- und ω-6-Fettsäuren sind Vorläufer von Gewebshormonen, den sogenannten Eicosanoiden wie den Prostaglandinen, die sich von der Dihomo-γ-linolensäure, der Arachidonsäure und der Eicosapentaensäure ableiten, und den Thromboxanen und Leukotrienen, die sich von der Arachidonsäure und der Eicosapentaensäure ableiten. Eicosanoide (sog. PG_2-Serie), die aus ω-6-Fettsäuren gebildet werden, fördern in der Regel Entzündungsreaktionen, während Eicosanoide (sog. PG_3-Serie) aus ω-3-Fettsäuren geringe oder keine entzündungsfördernde Wirkung haben.

Mehrfach ungesättigte langkettige ω-3-Fettsäuren wie Eicosapentaensäure (= EPA, $C20:5^{\Delta5,8,11,14,17}$) oder Docosahexaensäure (= DHA, $C22:6^{\Delta4,7,10,13,16,19}$) sind wichtige Komponenten der menschlichen Ernährung aufgrund ihrer verschiedenen Rollen in der Gesundheit, die Aspekte wie die Entwicklung des kindlichen Gehirns, die Funktionalität des Auges, die Synthese von Hormonen und anderer Signalstoffe, sowie die Vorbeugung von Herz-Kreislauf-Beschwerden, Krebs und Diabetes umfassen (Poulos, A (1995) Lipids 30:1-14; Horrocks, LA und Yeo YK (1999) Pharmacol Res 40:211-225).

Aufgrund der heute üblichen Zusammensetzung der menschlichen Nahrung ist ein Zusatz von mehrfach ungesättigten ω-3-Fettsäuren, die bevorzugt in Fischölen vorkommen, zur Nahrung besonders wichtig. So werden beispielsweise mehrfach ungesättigte

Fettsäuren wie Docosahexaensäure (= DHA, C22:6$^{\Delta 4,7,10,13,16,19}$) oder Eisosapentaensäure (= EPA, C20:5$^{\Delta 5,8,11,14,17}$) der Babynahrung zur Erhöhung des Nährwertes zugesetzt. Es besteht aus diesem Grund ein Bedarf an der Produktion mehrfach ungesättigter langkettiger Fettsäuren.

Hauptsächlich werden die verschiedenen Fettsäuren und Triglyceride aus Mikroorganismen wie Mortierella oder Schizochytrium oder aus Öl–produzierenden Pflanzen wie Soja, Raps und Algen wie Crypthecodinium oder Phaeodactylum und weiteren gewonnen, wobei sie in der Regel in Form ihrer Triacylglyceride (= Triglyceride = Triglycerole) anfallen. Sie können aber auch aus Tieren wie z.B. Fischen gewonnen werden. Die freien Fettsäuren werden vorteilhaft durch Verseifung der Triacylglyceride hergestellt. Sehr langkettige mehrfach ungesättigte Fettsäuren wie DHA, EPA, Arachidonsäure (ARA, C20:4$^{\Delta 5,8,11,14}$), Dihomo-γ-linolensäure (DHGL, C20:3$^{\Delta 8,11,14}$) oder Docosapentaensäure (DPA, C22:5$^{\Delta 7,10,13,16,19}$) werden in Ölfruchtpflanzen wie Raps, Soja, Sonnenblume, Färbersaflor jedoch nicht synthetisiert. Übliche natürliche Quellen für diese Fettsäuren sind Fische wie Hering, Lachs, Sardine, Goldbarsch, Aal, Karpfen, Forelle, Heilbutt, Makrele, Zander oder Thunfisch oder Algen.

Aufgrund der positiven Eigenschaften der mehrfach ungesättigten Fettsäuren hat es in der Vergangenheit nicht an Ansätzen gefehlt, Gene, die an der Synthese dieser Fettsäuren bzw. Triglyceride beteiligt sind, für die Herstellung von Ölen in verschiedenen Organismen mit geändertem Gehalt an ungesättigten Fettsäuren verfügbar zu machen. So wird in WO 91/13972 und seinem US–Äquivalent eine Δ–9–Desaturase beschrieben. In WO 93/11245 wird eine Δ–15–Desaturase und in WO 94/11516 eine Δ–12–Desaturase beansprucht. Weitere Desaturasen werden beispielsweise in EP–A–0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP–A–0 794 250, Stukey et al. (1990) J. Biol. Chem., 265: 20144-20149, Wada et al. (1990) Nature 347: 200–203 oder Huang et al. (1999) Lipids 34: 649–659 beschrieben. Die biochemische Charakterisierung der verschiedenen Desaturasen ist jedoch bisher nur unzureichend erfolgt, da die Enzyme als membrangebundene Proteine nur sehr schwer zu isolieren und zu charakterisieren sind (McKeon et al. (1981) Methods in Enzymol. 71: 12141–12147, Wang et al. (1988) Plant Physiol. Biochem., 26: 777–792).

In der Regel erfolgt die Charakterisierung membrangebundener Desaturasen durch Einbringung in einen geeigneten Organismus, der anschließend auf Enzymaktivität mittels Edukt- und Produktanalyse untersucht wird. Δ–6–Desaturasen werden in WO 93/06712, US 5,614,393, WO 96/21022, WO 00/21557 und WO 99/27111 beschrieben. Die Anwendung dieses Enzyms zur Produktion von Fettsäuren in transgenen Organismen wird in WO 98/46763, WO 98/46764 und WO 98/46765 beschrieben. Die Expression verschiedener Desaturasen und die Bildung mehrfach ungesättigter Fettsäuren wird auch in WO 99/64616 oder WO 98/46776 beschrieben und beansprucht. Bzgl. der Effektivität der Expression von Desaturasen und ihrem Einfluss auf die Bildung mehrfach ungesättigter Fettsäuren ist anzumerken, dass durch Expression einer einzelnen Desaturase wie bisher beschrieben lediglich geringe Gehalte an ungesättigten Fettsäuren/Lipiden wie z.B. γ-Linolensäure und Stearidonsäure erreicht wurden.

In der Vergangenheit wurden zahlreiche Versuche unternommen, Elongase-Gene zu erhalten. Millar and Kunst (1997) Plant Journal 12:121-131 und Millar et al. (1999) Plant Cell 11:825-838 beschreiben die Charakterisierung von pflanzlichen Elongasen zur Synthese von einfach ungesättigten langkettigen Fettsäuren (C22:1) bzw. zur Synthese von sehr langkettigen Fettsäuren für die Wachsbildung in Pflanzen (C_{28}-C_{32}). Beschreibungen zur Synthese von Arachidonsäure und EPA finden sich beispielsweise in WO 01/59128, WO 00/12720, WO 02/077213 und WO 02/08401. Die Synthese von mehrfach ungesättigter C24-Fettsäuren ist beispielsweise in Tvrdik et al. (2000) J. Cell Biol. 149: 707-718 oder in WO 02/44320 beschrieben.

Besonders geeignete Mikroorganismen zur Herstellung von PUFAs sind Mikroalgen wie Phaeodactylum tricornutum, Porphiridium-Arten, Thraustochytrien-Arten, Schizochytrien-Arten oder Crypthecodinium-Arten, Ciliaten, wie Stylonychia oder Colpidium, Pilze, wie Mortierella, Entomophthora oder Mucor und/oder Moose wie Physcomitrella, Ceratodon und Marchantia (R. Vazhappilly & F. Chen (1998) Botanica Marina 41: 553-558; K. Totani & K. Oba (1987) Lipids 22: 1060-1062; M. Akimoto et al. (1998) Appl. Biochemistry and Biotechnology 73: 269-278). Durch Stammselektion ist eine Anzahl von Mutantenstämmen der entsprechenden Mikroorganismen entwickelt worden, die eine Reihe wün-

schenswerter Verbindungen, einschließlich PUFAs, produzieren. Die Mutation und Selektion von Stämmen mit verbesserter Produktion eines bestimmten Moleküls wie den mehrfach ungesättigten Fettsäuren ist jedoch ein zeitraubendes und schwieriges Verfahren. Mit Hilfe der vorgenannten Mikroorganismen lassen sich zudem nur begrenzte Mengen der gewünschten mehrfach ungesättigten Fettsäuren wie DPA, EPA oder ARA herstellen, die noch dazu in der Regel als Fettsäuregemische anfallen. Deshalb werden, wann immer möglich, gentechnologische Verfahren bevorzugt.

Höhere Pflanzen enthalten mehrfach ungesättigte Fettsäuren wie Linolsäure (C18:2) und Linolensäure (C18:3). ARA, EPA und DHA kommen im Samenöl höherer Pflanzen gar nicht oder nur in Spuren vor (E. Ucciani: Nouveau Dictionnaire des Huiles Végétales. Technique & Documentation – Lavoisier, 1995. ISBN: 2-7430-0009-0). Es wäre jedoch vorteilhaft, in höheren Pflanzen, bevorzugt in Ölsaaten wie Raps, Lein, Sonnenblume und Soja, LCPUFAs herzustellen, da auf diese Weise große Mengen qualitativ hochwertiger LCPUFAs für die Lebensmittelindustrie, die Tierernährung und für pharmazeutische Zwecke kostengünstig gewonnen werden können. Hierzu werden vorteilhafterweise über gentechnische Methoden Gene, die für Enzyme der Biosynthese von LCPUFAs kodieren, in Ölsaaten eingeführt und exprimiert. Dies sind Gene, die beispielsweise für Δ-6-Desaturasen, Δ-6-Elongasen, Δ-5-Desaturasen oder Δ-4-Desaturasen kodieren. Diese Gene können vorteilhaft aus Mikroorganismen und niederen Pflanzen isoliert werden, die LCPUFAs herstellen und in den Membranen oder Triacylglyceriden einbauen. So konnten bereits Δ-6-Desaturase-Gene aus dem Moos Physcomitrella patens und Δ-6-Elongase-Gene aus P. patens und dem Nematoden C. elegans isoliert werden.

Transgene Pflanzen, die für Enzyme der LCPUFA-Biosynthese kodierende Gene enthalten und exprimieren und als Folge dessen LCPUFAs produzieren, wurden beispielsweise in DE-A-102 19 203 (Verfahren zur Herstellung mehrfach ungesättigter Fettsäuren in Pflanzen) beschrieben. Diese Pflanzen produzieren allerdings LCPUFAs in Mengen, die für eine Aufarbeitung der in den Pflanzen enthaltenen Öle noch weiter optimiert werden müssen. So beträgt der Gehalt von ARA in den in DE-A-102 19 203 beschriebenen Pflanzen lediglich 0,4 bis 2% und der Gehalt von EPA lediglich 0,5 bis 1%, jeweils bezogen auf den Gesamtlipidgehalt der Pflanze.

Um eine Anreicherung der Nahrung und des Futters mit mehrfach ungesättigten, langkettigen Fettsäuren zu ermöglichen, besteht daher ein großer Bedarf an einem einfachen, kostengünstigen Verfahren zur Herstellung von mehrfach ungesättigten, langkettigen Fettsäuren speziell in pflanzlichen Systemen.

Eine Aufgabe der Erfindung ist es daher, ein Verfahren bereitzustellen, mit dem langkettige, mehrfach ungesättigte Fettsäuren, insbesondere Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure, in transgenen Pflanzen in großer Menge preiswert hergestellt werden können.

Es wurde nun überraschenderweise herausgefunden, dass durch die Expression einer optimierten Δ5-Elongase-Sequenz in transgenen Pflanzen die Ausbeute an langkettigen, mehrfach ungesättigten Fettsäuren, insbesondere Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure, gesteigert werden kann.

Die durch das erfindungsgemäße Verfahren hergestellten PUFAs umfassen eine Gruppe von Molekülen, die höhere Tiere nicht mehr synthetisieren können und somit aufnehmen müssen oder die höhere Tiere nicht mehr ausreichend selbst herstellen können und somit zusätzlich aufnehmen müssen, obwohl sie leicht von anderen Organismen, wie Bakterien, synthetisiert werden können.

Entsprechend wird die Aufgabe der Erfindung gelöst durch das erfindungsgemäße Verfahren zur Herstellung von Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in einer transgenen Pflanze, umfassend das Bereitstellen in der Pflanze von mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Desaturase-Aktivität kodiert; und mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die

Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist. Für die Produktion von DHA muss desweiteren mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ4-Desaturase-Aktivität kodiert, in der Pflanze bereitgestellt werden.

Das "Bereitstellen in der Pflanze" bedeutet im Sinne der vorliegenden Erfindung, dass Maßnahmen getroffen werden, so dass die Nukleinsäuresequenzen kodierend für ein Polypeptid mit einer Δ6-Desaturase-Aktivität, ein Polypeptid mit einer Δ6-Elongase-Aktivität, ein Polypeptid mit einer Δ5-Desaturase-Aktivität und ein Polypeptid mit einer Δ5-Elongase-Aktivität zusammen in einer Pflanze vorliegen. Das "Bereitstellen in der Pflanze" umfasst somit das Einbringen der Nukleinsäuresequenzen in die Pflanze sowohl durch Transformation einer Pflanze mit einem oder mehreren rekombinanten Nukleinsäuremolekülen, die die genannten Nukleinsäuresequenzen enthalten, als auch durch Verkreuzung von geeigneten Elternpflanzen, die eine oder mehrere der genannten Nukleinsäuresequenzen enthalten.

Die Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, ist erfindungsgemäß gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist. Dies bedeutet, dass die Nukleinsäuresequenz gezielt für die Zwecke der Erfindung optimiert wurde, ohne dass dadurch die von der Nukleinsäuresequenz kodierte Aminosäuresequenz verändert wurde.

Der genetische Code ist redundant, da er 61 Kodons verwendet, um 20 Aminosäuren zu spezifizieren. Daher werden die meisten der 20 proteinogenen Aminosäuren von mehreren Tripletts (Kodons) kodiert. Die synonymen Kodons, die eine einzelne Aminosäure spezifizieren, werden in einem bestimmten Organismus jedoch nicht mit gleicher Häufigkeit verwendet, sondern es gibt bevorzugte Kodons, die häufig verwendet werden und Kodons, die seltener verwendet werden. Diese Unterschiede in der Kodonverwendung werden zurückgeführt auf selektive evolutionäre Drücke und vor allem die Effizienz der Translation. Ein Grund für die geringere Translationseffizienz von selten auftretenden

Kodons könnte darin liegen, dass die entsprechenden Aminoacyl-tRNA-Pools erschöpft werden und damit nicht mehr zur Proteinsynthese zur Verfügung stehen.

Außerdem bevorzugen unterschiedliche Organismen unterschiedliche Kodons. Daher läuft beispielsweise die Expression einer rekombinanten DNA, die aus einer Säugerzelle stammt, in E. coli-Zellen häufig nur suboptimal ab. Deshalb kann der Austausch selten verwendeter Kodons gegen häufig verwendete Kodons in manchen Fällen die Expression erhöhen. Ohne an eine Hypothese gebunden sein zu wollen, wird angenommen, dass die kodonoptimierten DNA-Sequenzen eine effizientere Translation ermöglichen und die daraus gebildeten mRNAs möglicherweise eine höhere Halbwertszeit in der Zelle besitzen und daher häufiger für die Translation zur Verfügung stehen. Aus dem vorstehend gesagten folgt, dass eine Kodonoptimierung nur dann nötig ist, wenn der Organismus, in dem die Nukleinsäuresequenz exprimiert werden soll, ein anderer ist als der Organismus, aus dem die Nukleinsäuresequenz ursprünglich stammt.

Für viele Organismen, von denen die DNA-Sequenz einer größeren Zahl von Genen bekannt ist, gibt es Tabellen, denen man die Häufigkeit der Verwendung bestimmter Kodons in dem jeweiligen Organismus entnehmen kann. Mit Hilfe dieser Tabellen lassen sich Proteinsequenzen mit relativ großer Genauigkeit in eine DNA-Sequenz zurückübersetzen, die die im jeweiligen Organismus bevorzugten Kodons für die verschiedenen Aminosäuren des Proteins enthält. Tabellen zur Kodonverwendung können u.a. unter der folgenden Internet-Adresse aufgefunden werden: http://www.kazusa.or.jp/Kodon/E.html. Darüber hinaus bieten mehrere Firmen Software für die Genoptimierung an, wie z.B. die Firma Entelechon (Software Leto) oder die Firma Geneart (Software GeneOptimizer).

Die Anpassung der Sequenzen an die Kodonverwendung in einem bestimmten Organismus kann unter Zuhilfenahme verschiedener Kriterien erfolgen. Zum einen kann für eine bestimmte Aminosäure immer das am häufigsten im ausgewählten Organismus vorkommende Kodon verwendet werden, zum anderen kann aber auch die natürliche Frequenz der verschiedenen Kodons berücksichtigt werden, so dass alle Kodons für eine bestimmte Aminosäure entsprechend ihrer natürlichen Häufigkeit in die optimierte

Sequenz eingebaut werden. Dabei kann die Auswahl, an welcher Position welches Basen-Triplett verwendet wird, zufällig stattfinden. Erfindungsgemäß wurde die DNA-Sequenz unter Berücksichtigung der natürlichen Häufigkeit einzelner Kodons angepasst, wobei die Verwendung des am häufigsten im ausgewählten Organismus vorkommenden Kodons ebenfalls geeignet ist.

Besonders bevorzugt ist eine Nukleinsäuresequenz aus Ostreococcus tauri, die für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, wie beispielsweise das in Seq ID No. 110 dargestellte Polypeptid, zumindest an die Kodonverwendung in Raps, Soja und/oder Lein angepasst. Bei der ursprünglich aus Ostreococcus tauri stammenden Nukleinsäuresequenz handelt es sich bevorzugt um die in Seq ID No. 109)dargestellte Sequenz. Die für die Δ5-Elongase kodierende DNA-Sequenz ist an mindestens 20% der Positionen, bevorzugt an mindestens 30% der Positionen, besonders bevorzugt an mindestens 40% Positionen und am meisten bevorzugt an mindestens 50% der Positionen an die Kodonverwendung in Raps, Soja und/oder Lein angepasst.

Am meisten bevorzugt handelt es sich bei der verwendeten Nukleinsäuresequenz um die in SEQ ID No. 64 angegebene Sequenz.

Es versteht sich, dass auch solche kodonoptimierten DNA-Sequenzen von der Erfindung erfasst sind, die für ein Polypeptid mit der Aktivität einer Δ5-Elongase kodieren, dessen Aminosäuresequenz an einer oder mehreren Positionen gegenüber der Wildtyp-Sequenz verändert ist, das aber noch im wesentlichen die gleiche Aktivität aufweist wie das Wildtyp-Protein.

Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert, ausgewählt aus der Gruppe bestehend aus:
a) Nukleinsäuresequenzen mit der in Seq ID NO. 1,3,5,7,9,11,13,15,17,19,21,23,25,27,29,31,33,35,37,39 oder 41, bevorzugt mit der in Seq ID No. 1 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die für die in Seq ID No. 2,4,6,8,10,12,14,16,18,20,22,24,26,28,30,32,34,36,38,40 oder 42, bevorzugt in Seq ID No. 2 angegebene Aminosäuresequenz kodieren,

c) Nukleinsäuresequenzen, die mit dem komplementären Strang der a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbesondere der in Seq ID No. 1 angegebenen Nukleinsäuresequenz unter stringenten Bedingungen hybridisieren,

d) Nukleinsäuresequenzen, die zu den in a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbeondere zur der in Seq ID No. 1 angegebenen Sequenz zu mindestens 60%, 65%, 70%, 75% oder 80%, bevorzugt zu mindestens 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% oder 90%, besonders bevorzugt zu mindestens 91%, 92%, 93%, 94% oder 95% und insbesondere zu mindestens 96%, 97%, 98% oder 99% identisch sind, und

e) Nukleinsäuresequenzen, die für eine Aminosäuresequenz kodieren, die mindestens eines, beispielsweise 2, 3, 4, 5, 6, 7 oder 8, vorzugsweise alle der in Seq ID No. 43,44,45,46,47,48,49 oder 50 angegebenen Aminosäurepattern aufweisen.

Unter Aminosäurepattern sind kurze Aminosäuresequenzen zu verstehen, die vorzugsweise weniger als 50, besonders bevorzugt weniger als 40 und insbesondere von 10 bis 40 und noch weiter bevorzugt von 10 bis 30 Aminosäuren umfassen.

Für die vorliegende Erfindung wird die Identität vorzugsweise über die Vollänge der erfindungsgemäßen Nukleotid- oder Aminosäuresequenzen ermittelt, beispielsweise für die in SEQ ID NO: 64 angegebene Nukleinsäuresequenz über die Vollänge von 903 Nukleotiden.

Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert, ausgewählt aus der Gruppe bestehend aus:

a) Nukleinsäuresequenzen mit der in Seq ID No. 171, 173,175,177,179,181 oder 183, insbesondere mit der in Seq ID No. 171 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die für die in Seq ID No. 172, 174,176,178,180,182 oder184, insbesondere für die in Seq ID No. 172 angegebene Aminosäuresequenz kodieren,

c) Nukleinsäuresequenzen, die mit dem komplementären Strang der a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbesondere der in Seq ID No. 1 angegebenen Nukleinsäuresequenz unter stringenten Bedingungen hybridisieren,

d) Nukleinsäuresequenzen, die zu den in a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbesondere zu der in Seq ID No. 171 angegebenen Sequenz zu mindestens 60%, 65%, 70%, 75% oder 80%, bevorzugt zu mindestens 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% oder 90%, besonders bevorzugt zu mindestens 91%, 92%, 93%, 94% oder 95% und insbesondere zu mindestens 96%, 97%, 98% oder 99% identisch sind, und

e) Nukleinsäuresequenzen, die für eine Aminosäuresequenz kodieren, die mindestens eines, beispielsweise 2, 3, 4, 5, 6, 7 oder 8, vorzugsweise alle der in Seq ID No. 185,186,187,188,189,190,191 oder192 angebebenen Aminosäurepattern aufweisen.

Insbesondere handelt es sich bei der Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert ebenfalls um eine nach der vorliegenden Erfindung kodonoptimierten Sequenz, bevorzugt um die in der SEQ ID NO: 122 darstellte Nukleinsäuresequenz.

Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ5-Desaturase-Aktivität kodiert, ausgewählt aus der Gruppe bestehend aus:

a) Nukleinsäuresequenzen mit der in Seq ID No. 51, 53 oder 55, bevorzugt mit der in Seq ID No. 51 dargestellten Sequenz,

b Nukleinsäuresequenzen, die für die in Seq ID No. 52, 54 oder 56, bevorzugt mit der in Seq ID No. 52 angegebenen Aminosäuresequenz kodieren,

c) Nukleinsäuresequenzen, die mit dem komplementären Strang der in a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbesondere der in Seq ID No. 51 angegebenen Nukleinsäuresequenz unter stringenten Bedingungen hybridisieren, und

d) Nukleinsäuresequenzen, die zu den in a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbesondere zur der in Seq ID No. 51 angegebenen Sequenz zu mindestens 60%, 65%, 70%, 75% oder 80%, bevorzugt zu mindestens 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89% oder 90%, besonders bevorzugt zu mindestens 91%, 92%, 93%, 94% oder 95% und insbesondere zu mindestens 96%, 97%, 98% oder 99% identisch sind, und

e) Nukleinsäuresequenzen, die für eine Aminosäuresequenz kodieren, die mindestens eines, beispielsweise 2, 3, 4, 5, 6 oder 7 , vorzugsweise alle der in Seq ID No. 57,58,59,60,61,62 oder 63 angebebenen Aminosäurepattern aufweisen.

Weitere geeignete Nukleinsäuresequenzen kann der Fachmann der Literatur bzw. den bekannten Genbanken wie z.B. http://www.ncbi.nlm.nih.gov entnehmen.

In einer weiteren bevorzugten Ausführungsform des Verfahrens werden zusätzlich eine oder mehrere Nukleinsäuresequenzen, die für ein Polypeptid mit der Aktivität einer ω-3-Desaturase und/oder einer Δ4-Desaturase kodieren, in die Pflanze eingebracht.

Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer ω-3-Desaturase-Aktivität kodiert, ausgewählt aus der Gruppe bestehend aus:

a) Nukleinsäuresequenzen mit der in Seq ID No. 193 oder 195, vorzugsweise der in Seq ID No. 193 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die für die in Seq ID No. 194 angegebene Aminosäuresequenz kodieren,

c) Nukleinsäuresequenzen, die mit dem komplementären Strang der in Seq ID No. 193 oder 195 angegebenen Nukleinsäuresequenz unter stringenten Bedingungen hybridisieren, und

d) Nukleinsäuresequenzen, die zu der in Seq ID No. 193 oder 195 angegebenen Sequenz zu mindestens 60%, 65%, 70%, 75% oder 80%, bevorzugt zu mindestens 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% oder 90%, besonders bevorzugt zu mindestens 91%, 92%, 93%, 94% oder 95% und insbesondere zu mindestens 96%, 97%, 98% oder 99% identisch sind.

Die im erfindungsgemäßen Verfahren vorteilhaft verwendete ω-3-Desaturase ermöglicht eine Verschiebung vom ω-6-Biosyntheseweg zum ω-3-Biosyntheseweg, was zu einer Verschiebung von $C_{18:2}$- zu $C_{18:3}$-Fettsäuren führt. Weiterhin ist vorteilhaft, dass die ω-3-

Desaturase eine breite Palette von Phospholipiden wie Phosphatidylcholin (= PC), Phosphatidylinositol (= PIS) oder Phosphatidylethanolamin (= PE) umsetzt. Schließlich lassen sich auch Desaturierungsprodukte in den Neutrallipiden (= NL), das heißt in den Triglyceriden finden.

Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ4-Desaturase-Aktivität kodiert, ausgewählt aus der Gruppe bestehend aus:

a) Nukleinsäuresequenzen mit der in Seq ID No. 77, 79,81,83,85,87,89,91 oder 93, bevorzugt mit der in der Seq ID No. 77 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die für die in Seq ID No. 78, 80,82,84,86,88,90,92 oder 94, bevorzugt für die in Seq ID No. 78 angegebene Aminosäuresequenz kodieren,

c) Nukleinsäuresequenzen, die mit dem komplementären Strang der in a) oder b) oberhalb angegebenen Nukleinsäuresequenzen, insbesondere der in Seq ID No. 77 angegebenen Nukleinsäuresequenz unter stringenten Bedingungen hybridisieren, und

d) Nukleinsäuresequenzen, die zu der in Seq ID No. 77 angegebenen Sequenz zu mindestens 60%, 65%, 70%, 75% oder 80%, bevorzugt zu mindestens 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% oder 90%, besonders bevorzugt zu mindestens 91%, 92%, 93%, 94% oder 95% und insbesondere zu mindestens 96%, 97%, 98% oder 99% identisch sind, und

e) Nukleinsäuresequenzen, die für eine Aminosäuresequenz kodieren, die mindestens eines, beispielsweise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 oder 14, vorzugsweise alle der in Seq ID No. 95,96,97,98,99,100,101,102,103,104,105,106,107 oder 108 angebebenen Aminosäurepattern aufweisen.

Die Δ4-Desaturase, die im erfindungsgemäßen Verfahren vorteilhaft verwendet wird, katalysiert die Einführung einer Doppelbindung in die Fettsäure Docosapentaensäure, was zur Bildung von Docosahexaensäure führt.

Für das erfindungsgemäße beschriebene Verfahren ist es vorteilhaft, in die Pflanzen zusätzlich zu den Nukleinsäuresequenzen, die für Polypeptide mit einer Δ6-Desaturase-Aktivität, einer Δ6-Elongase-Aktivität, einer Δ5-Desaturase-Aktivität und einer Δ5-

Elongase-Aktivität kodieren, sowie den ggf. eingebrachten Nukleinsäuresequenzen, die für ein Polypeptid mit einer ω-3-Desaturase-Aktivität und/oder einer Δ4-Desaturase-Aktivität kodieren, zusätzlich weitere Nukleinsäuren einzubringen, die für Enzyme des Fettsäure- oder Lipidstoffwechsels kodieren.

Im Prinzip können alle Gene des Fettsäure– oder Lipidstoffwechsels in Kombination mit den im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen verwendet werden; bevorzugt werden Gene des Fettsäure– oder Lipidstoffwechsels ausgewählt aus der Gruppe Acyl-CoA-Dehydrogenase(n), Acyl-ACP(= acyl carrier protein)–Desaturase(n), Acyl–ACP–Thioesterase(n), Fettsäure–Acyl–Transferase(n), Acyl-CoA:Lysophospholipid-Acyltransferasen, Fettsäure–Synthase(n), Fettsäure–Hydroxylase(n), Acetyl-Coenzym A–Carboxylase(n), Acyl–Coenzym A–Oxidase(n), Fettsäure–Desaturase(n), Fettsäure–Acetylenasen, Lipoxygenasen, Triacylglycerol–Lipasen, Allenoxid–Synthasen, Hydroperoxid–Lyasen oder Fettsäure–Elongase(n) in Kombination mit der Δ-6-Elongase, Δ-6-Desaturase, Δ-5-Desaturase und der Δ-5-Elongase sowie ggf. der ω-3-Desaturase und/oder der Δ-4-Desaturase verwendet, wobei einzelne Gene oder mehrere Gene in Kombination verwendet werden können.

Vorteilhaft werden die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuren im vegetativen Gewebe (= somatischem Gewebe) exprimiert. Unter vegetativem Gewebe ist im Sinne dieser Erfindung ein Gewebe zu verstehen, das sich durch mitotische Teilungen vermehrt. Derartiges Gewebe entsteht auch durch asexuelle Fortpflanzung (= Apomixis) und Vermehrung. Von Vermehrung spricht man dann, wenn sich die Zahl der Individuen in aufeinander folgenden Generationen erhöht. Diese durch asexuelle Vermehrung entstandenen Individuen sind mit ihren Eltern weitestgehend identisch. Beispiele für derartige Gewebe sind Blatt, Blüte, Wurzel, Stengel, oberirdische oder unterirdische Ausläufer (Seitensprosse, Stolonen), Rhizome, Knospen, Knollen wie Wurzelknollen oder Ausläuferknollen, Zwiebel, Brutkörper, Brutknospen, Bulbillen oder Turione. Derartige Gewebe können auch durch unechte, echte oder durch den Mensch verursachte Viviparie entstehen. Aber auch Samen, die durch Agamospermie, wie sie für Asteraceae, Poaceae oder Rosaceae typisch sind, entstanden sind, gehören zu den vegetativen Geweben, in denen vorteilhaft die Expression stattfindet. Zu einem geringeren Teil oder gar

nicht werden die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuren im generativen Gewebe (Keimbahngewebe) exprimiert. Beispiele für derartige Gewebe sind Gewebe, die durch geschlechtliche Fortpflanzung, d.h. meiotische Zellteilungen entstehen, wie z.B. Samen, die durch geschlechtliche Prozesse entstanden sind. Unter zu einem geringen Teil ist zu verstehen, dass im Vergleich zum vegetativen Gewebe die Expression gemessen auf RNA- und/oder Proteinebene weniger als 5 %, vorteilhaft weniger als 3 %, besonders vorteilhaft weniger als 2 %, am meisten bevorzugt weniger als 1; 0,5; 0,25 oder 0,125 % beträgt.

Besonders bevorzugt werden die Nukleinsäuresequenzen in den Blättern der transgenen Pflanzen exprimiert. Dies hat den Vorteil, dass die erfindungsgemäß hergestellten LCPUFAs von Tieren und Menschen direkt durch den Verzehr der Blätter aufgenommen werden können und keine vorherige Aufarbeitung des Pflanzenmaterials erforderlich ist.

Die Expression der erfindungsgemäßen Nukleinsäuresequenzen im Blatt kann durch die Verwendung von konstitutiven oder blattspezifischen Promotoren erreicht werden.

"Konstitutive Promotoren" sind Promotoren, die die Expression in einer Vielzahl, vorzugsweise in allen, Geweben über einen wesentlichen Zeitraum während der pflanzlichen Entwicklung, bevorzugt während der gesamten pflanzlichen Entwicklung, ermöglichen. Bevorzugt wird ein Promotor aus einer Pflanze oder aus einem Pflanzenvirus verwendet. Bevorzugt sind der Promotor des CaMV (cauliflower mosaic virus) 35S-Transkripts (Franck et al. (1980) Cell 21: 285-294), der 19S CaMV-Promotor (US 5,352,605), der Aktinpromotor aus Reis (McElroy et al. (1990) Plant Cell 2: 163-171), der Legumin B-Promotor (GenBank Acc. No. X03677), der Promotor der Nopalinsynthase aus Agrobacterium, der TR duale Promotor, der Octopinsynthase-Promotor aus Agrobacterium, der Ubiquitin-Promotor (Holtorf et al. (1995) Plant Mol. Biol. 29: 637-649), der Smas-Promotor, der Cinnamoylalkoholdehydrogenase-Promotor (US 5,683,439), die Promotoren der vacuolaren ATPase-Untereinheiten, der pEMU-Promotor (Last et al. (1991) Theor. Appl. Genet. 81: 581-588), der MAS-Promotor (Velten et al. (1984) EMBO J. 3(12): 2723-2730), der Histon-H3-Promotor aus Mais (Lepetit et al. (1992) Mol. Gen. Genet. 231: 276-285), der Promotor des Nitrilase 1-Gens aus Arabidopsis (GenBank

Acc. No. U38846, Nukleotide 3862-5325) und der Promotor eines Prolin-reichen Proteins aus Weizen (WO 91/13991) und weitere Promotoren, die konstitutive Genexpression vermitteln. Besonders bevorzugt ist der Promotor des CaMV 35S-Transkripts.

Es ist im Prinzip möglich, alle natürlich auftretenden konstitutiven Promotoren mit ihren Regulationssequenzen, wie die oben genannten, für das neue Verfahren zu verwenden. Es ist aber ebenfalls möglich, zusätzlich oder alleine synthetische Promotoren zu verwenden.

"Blattspezifische Promotoren" sind Promotoren, die eine hohe Aktivität im Blatt und keine oder nur eine geringe Aktivität in anderen Geweben zeigen. Unter "geringer Aktivität" wird im Rahmen der Erfindung verstanden, dass die Aktivität in anderen Geweben weniger als 20%, bevorzugt weniger als 10%, besonders bevorzugt weniger als 5% und am meisten bevorzugt weniger als 3, 2 oder 1% der Aktivität im Blatt beträgt. Geeignete blattspezifische Promotoren sind z.B. die Promotoren der kleinen Untereinheit von Rubisco (Timko et al. (1985) Nature 318: 579-582) und des Chlorophyll a/b-bindenden Proteins (Simpson et al. (1985) EMBO J. 4: 2723-2729).

Dem Fachmann sind weitere blattspezifische Promotoren bekannt bzw. er kann mit bekannten Methoden weitere geeignete Promotoren zu isolieren. So kann der Fachmann mit Hilfe gängiger molekularbiologischer Methoden, z. B. Hybridisierungsexperimenten oder DNA-Protein-Bindungsstudien, Blatt-spezifische regulatorische Nukleinsäureelemente identifizieren. Dabei wird z. B. in einem ersten Schritt aus Blattgewebe des gewünschten Organismus, aus dem die regulatorischen Sequenzen isoliert werden sollen, die gesamte poly(A)$^+$-RNA isoliert und eine cDNA-Bank angelegt. In einem zweiten Schritt werden mit Hilfe von cDNA-Klonen, die auf poly(A)$^+$-RNA-Molekülen aus einem Nicht-Blattgewebe basieren, aus der ersten Bank mittels Hybridisierung diejenigen Klone identifiziert, deren korrespondierende poly(A)$^+$-RNA-Moleküle lediglich im Blattgewebe akkumulieren. Anschließend werden mit Hilfe dieser so identifizierten cDNAs Promotoren isoliert, die über Blatt-spezifische regulatorische Elemente verfügen. Dem Fachmann stehen darüber hinaus weitere auf PCR basierende Methoden für die Isolierung geeigneter Blatt-spezifischer Promotoren zur Verfügung.

Selbstverständlich können die Nukleinsäuresequenzen der vorliegenden Erfindung auch in den Samen der transgenen Pflanzen exprimiert werden, indem samen-spezifische Promotoren verwendet werden, die im Embryo und/oder im Endosperm aktiv sind. Samen-spezifische Promotoren können prinzipiell sowohl aus dikotyledonen als auch aus monokotyledonen Pflanzen isoliert werden. Im Folgenden sind bevorzugte Promotoren aufgeführt: USP (= unknown seed protein) und Vicilin (Vicia faba) (Bäumlein et al. (1991) Mol. Gen Genet. 225(3): 459-467), Napin (Raps) (US 5,608,152), Conlinin (Lein) (WO 02/102970), Acyl-Carrier Protein (Raps) (US 5,315,001 und WO 92/18634), Oleosin (Arabidopsis thaliana) (WO 98/45461 und WO 93/20216), Phaseolin (Phaseolus vulgaris) (US 5,504,200), Bce4 (WO 91/13980), Leguminosen B4 (LegB4-Promotor) (Bäumlein et al. (1992) Plant J. 2(2): 233-239), Lpt2 und lpt1(Gerste) (WO 95/15389 und WO95/23230), Samen-spezifische Promotoren aus Reis, Mais und Weizen (WO 99/16890), Amy32b, Amy 6-6 und Aleurain (US 5,677,474), Bce4 (Raps) (US 5,530,149), Glycinin (Soja) (EP 571 741), Phosphoenol-Pyruvatcarboxylase (Soja) (JP 06/62870), ADR12-2 (Soja) (WO 98/08962), Isocitratlyase (Raps) (US 5,689,040) oder α-Amylase (Gerste) (EP 781 849).

In einer besonders bevorzugten Ausführungsform der vorliegenden Erfindung werden die verwendeten Nukleinsäuresequenzen, insbesondere die für eine Δ-5 Elongase kodierende Nukleinsäuresequenz, welche gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist, bevorzugt die in SEQ ID NO: 64 beschriebene Nukleinsäuresequenz im generativen Gewebe, insbesondere im Samen exprimiert. Die spezifische Expression im Samen erfolgt vorteilhaft unter Verwendung eines der oben erwähnten samenspezifischen Promotoren, insbesondere unter Verwendung des Napin Promotors. In dieser besonders bevorzugten Ausführungsform beträgt der Gehalt an den hergestellten LCPUFAs, insbesondere an den C22 Fettsäuren im Samenöl mindestens 5 Gew.-%, vorteilhaft mindestens 6, 7, 8, 9 oder 10 Gew.-%, bevorzugt von mindestens 11, 12, 13, 14 oder 15 Gew.-%, besonders bevorzugt von mindestens 16, 17, 18, 19, oder 20 Gew.-%, ganz besonders bevorzugt von mindestens 25, 30, 35 oder 40 Gew.-% des Samenölgehalt. In einer weiteren besonders bevorzug-

ten Ausführungsform mit der in SEQ ID NO: 63 beschriebenen Nukleinsäuresequenz beträgt der Gehalt an C22-Fettsäuren im Samenöl mindestens 8 Gew.-% des Samenölgehalts.

In einer weiteren besonders bevorzugten Ausführungsform der vorliegenden Erfindung werden die verwendeten Nukleinsäuresequenzen, insbesondere die für eine Δ-5 Elongase kodierende Nukleinsäuresequenz, welche gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist, bevorzugt die in SEQ ID NO: 64 beschriebene Nukleinsäuresequenz im generativen Gewebe, insbesondere im Samen exprimiert. Die spezifische Expression im Samen erfolgt vorteilhaft unter Verwendung eines der oben erwähnten samenspezifischen Promotoren, insbesondere unter Verwendung des Napin-Promotors. In dieser besonders bevorzugten Ausführungsform beträgt der Gehalt an Docosahexaensäure im Samenöl mindestens 1 Gew.-%, bevorzugt mindestens 1,1, 1,2, 1,3, 1,4 oder 1,5 Gew.-%, besonders bevorzugt mindestens 1,6, 1,7, 1,8 oder 1,9 Gew.-%, insbesondere mindestens 2, 2,1, 2,2, 2,3, 2,4, 2,5, 2,6, 2,7, 2,8 oder 2,9 Gew.-%, weiterhin bevorzugt mindestens 3, 3,5 oder 4 Gew.-% des Samenölgehalts. In einer weiteren besonders bevorzugten Ausführungsform mit der in SEQ ID NO: 63 beschriebenen Nukleinsäuresequenz beträgt der Gehalt an Docosahexaensäure im Samenöl mindestens 1,9 Gew.-% des Samenölgehalts. Es ist dem Fachmann dabei bekannt, dass zur Herstellung von Docosahexaensäure zusätzlich eine oder mehrere Nukleinsäuresequenzen, die für ein Polypeptid mit der Aktivität einer Δ4-Desaturase-Aktivität kodiert, benötigt werden. Vorteilhaft wird eine Nukleinsäuresequenz, die für ein Polypeptid mit der Aktivität einer Δ4-Desaturase-Aktivität kodiert, ausgewählt aus der Gruppe bestehend aus Nukleinsäuresequenzen mit der in Seq ID No. 77, 79,81,83,85,87,89,91 oder 93, bevorzugt mit der in der Seq ID No. 77 dargestellten Sequenz.

In einer weiteren besonders bevorzugten Ausführungsform der vorliegenden Erfindung werden die verwendeten Nukleinsäuresequenzen, insbesondere die für eine Δ-5 Elongase kodierende Nukleinsäuresequenz, welche gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die

Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist, bevorzugt die in SEQ ID NO: 64 beschriebene Nukleinsäuresequenz im generativen Gewebe, insbesondere im Samen exprimiert. Die spezifische Expression im Samen erfolgt vorteilhaft unter Verwendung eines der oben erwähnten samenspezifischen Promotoren, insbesondere unter Verwendung des NapinPromotors. In dieser besonders bevorzugten Ausführungsform beträgt der Gehalt an Docosahexaensäure im Samenöl mindestens 1 Gew.-%, bevorzugt mindestens 1,1, 1,2, 1,3, 1,4 oder 1,5 Gew.-%, besonders bevorzugt mindestens 1,6, 1,7, 1,8 oder 1,9 Gew.-%, insbesondere mindestens 2, 2,1, 2,2, 2,5, 2,6, 2,7, 2,8 oder 2,9 Gew.-%, weiterhin bevorzugt mindestens 3, 3,5, oder 4 Gew.-% des Samenölgehalts. Dabei beträgt der Gehalt an den hergestellten LCPUFAs, insbesondere an den C22 Fettsäuren im Samenöl mindestens 5 Gew.-%, vorteilhaft mindestens 6, 7, 8, 9 oder 10 Gew.-%, bevorzugt von mindestens 11, 12, 13, 14 oder 15 Gew.-%, besonders bevorzugt von mindestens 16, 17, 18, 19, oder 20 Gew.-%, ganz besonders bevorzugt von mindestens 25, 30, 35 oder 40 Gew.-% des Samenölgehalt. In einer weiteren besonders bevorzugten Ausführungsform mit der in SEQ ID NO: 63 beschriebenen Nukleinsäuresequenz beträgt der Gehalt an Docosahexaensäure im Samenöl mindestens 1,9 Gew.-% des Samenölgehalts, wobei der Gehalt an C22-Fettsäuren im Samenöl mindestens 8 Gew.-% des Samenölgehalts beträgt.

Die Pflanzengenexpression lässt sich auch über einen chemisch induzierbaren Promotor erleichtern (siehe eine Übersicht in Gatz (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemisch induzierbare Promotoren eignen sich besonders, wenn gewünscht wird, dass die Genexpression auf zeitspezifische Weise erfolgt. Beispiele für solche Promotoren sind ein Salicylsäure-induzierbarer Promotor (WO 95/19443), ein Tetracyclin-induzierbarer Promotor (Gatz et al. (1992) Plant J. 2, 397-404) und ein Ethanol-induzierbarer Promotor.

Auch Promotoren, die auf biotische oder abiotische Stressbedingungen reagieren, sind geeignete Promotoren, wie beispielsweise der pathogeninduzierte PRP1-Gen-Promotor (Ward et al. (1993) Plant. Mol. Biol. 22: 361-366), der hitzeinduzierbare hsp80-Promotor aus Tomate (US 5,187,267), der kälteinduzierbare Alpha-Amylase-Promotor aus Kartof-

fel (WO 96/12814) oder der durch Wunden induzierbare pinII-Promotor (EP-A-0 375 091).

Ebenfalls besonders geeignet sind Promotoren, welche die plastidenspezifische Expression herbeiführen, da Plastiden das Kompartiment sind, in dem die Vorläufer sowie einige Endprodukte der Lipidbiosynthese synthetisiert werden. Geeignete Promotoren, wie der virale RNA-Polymerase-Promotor, sind beschrieben in WO 95/16783 und WO 97/06250, und der clpP-Promotor aus Arabidopsis, beschrieben in WO 99/46394.

Es versteht sich, dass die erfindungsgemäß hergestellten mehrfach ungesättigten Fettsäuren nicht nur in intakten transgenen Pflanzen produziert werden können, sondern auch in pflanzlichen Zellkulturen oder in Kalluskulturen.

Die im Verfahren hergestellten mehrfach ungesättigten Fettsäuren sind vorteilhaft in Phospholipiden und/oder Triacylglyceriden gebunden, können aber auch als freie Fettsäuren oder aber gebunden in Form anderer Fettsäureester in den Organismen vorkommen. Dabei können sie als “Reinprodukte“ oder aber vorteilhaft in Form von Mischungen verschiedener Fettsäuren oder Mischungen unterschiedlicher Phospholipide wie Phosphatidylglycerol, Phosphatidylcholin, Phosphatidylethanolamin und/oder Phosphatidylserin und/oder Triacylglyceriden, Monoacylglyceriden und/oder Diacylglyceriden vorliegen. Vorteilhaft liegen die im Verfahren hergestellten LCPUFAS EPA, DPA und DHA im Phosphatidylcholin und/oder Phosphatidylethanolamin und/oder in den Triacylglyceriden vor. Die Triacylglyceride können außerdem noch weitere Fettsäuren enthalten wie kurzkettige Fettsäuren mit 4 bis 6 C-Atomen, mittelkettige Fettsäuren mit 8 bis 12 C-Atomen oder langkettige Fettsäuren mit 14 bis 24 C-Atomen. Bevorzugt enthalten sie langkettige Fettsäuren, besonders bevorzugt C_{20}- oder C_{22}-Fettsäuren.

Unter dem Begriff “Glycerid” wird ein mit ein, zwei oder drei Carbonsäureresten verestertes Glycerin verstanden (Mono-, Di- oder Triglycerid). Unter “Glycerid” wird auch ein Gemisch aus verschiedenen Glyceriden verstanden. Vorteilhaft handelt es sich bei dem Glycerid um ein Triglycerid. Das Glycerid oder das Glyceridgemisch kann weitere Zusät-

ze, z.B. freie Fettsäuren, Antioxidantien, Proteine, Kohlenhydrate, Vitamine und/oder andere Substanzen enthalten.

Unter einem "Glycerid" im Sinne des erfindungsgemäßen Verfahrens werden ferner vom Glycerin abgeleitete Derivate verstanden. Dazu zählen neben den oben beschriebenen Fettsäureglyceriden auch Glycerophospholipide und Glyceroglycolipide. Bevorzugt seien hier die Glycerophospholipide wie Lecithin (Phosphatidylcholin), Cardiolipin, Phosphatidylglycerin, Phosphatidylserin und Alkylacylglycerophospholipide beispielhaft genannt.

Unter Phospholipiden im Sinne der Erfindung sind zu verstehen Phosphatidylcholin, Phosphatidylethanolamin, Phosphatidylserin, Phosphatidylglycerin und/oder Phosphatidylinositol.

Die Fettsäureester mit mehrfach ungesättigten C_{18}–, C_{20}- und/oder C_{22}-Fettsäuremolekülen können aus den Nutzpflanzen, die für die Herstellung der Fettsäureester verwendet wurden, in Form eines Öls oder Lipids beispielsweise in Form von Verbindungen wie Sphingolipiden, Phosphoglyceriden, Lipiden, Glycolipiden wie Glycosphingolipiden, Phospholipiden wie Phosphatidylethanolamin, Phosphatidylcholin, Phosphatidylserin, Phosphatidylglycerol, Phosphatidylinositol oder Diphosphatidylglycerol, Monoacylglyceriden, Diacylglyceriden, Triacylglyceriden oder sonstigen Fettsäureestern wie den Acetyl-CoenzymA-Estern, die die mehrfach ungesättigten Fettsäuren mit mindestens zwei, drei oder vier, bevorzugt vier, fünf oder sechs Doppelbindungen enthalten, isoliert werden, vorteilhaft werden sie in der Form ihrer Diacylglyceride, Triacylglyceride und/oder in Form des Phosphatidylesters isoliert, besonders bevorzugt in der Form der Triacylglyceride, Phosphatidylcholin und/oder Phosphatidylethanolamin. Neben diesen Estern sind die mehrfach ungesättigten Fettsäuren auch als freie Fettsäuren oder gebunden in anderen Verbindungen in den Pflanzen enthalten. In der Regel liegen die verschiedenen vorgenannten Verbindungen (Fettsäureester und freie Fettsäuren) in den Organismen in einer ungefähren Verteilung von 80 bis 90 Gew.-% Triglyceride, 2 bis 5 Gew.-% Diglyceride, 5 bis 10 Gew.-% Monoglyceride, 1 bis 5 Gew.-% freie Fettsäuren, 2 bis 8 Gew.-% Phospholipide vor, wobei sich die Summe der verschiedenen Verbindungen zu 100 Gew.-% ergänzt.

Im erfindungsgemäßen Verfahren werden die hergestellten LCPUFAs mit einem Gehalt von mindestens 4 Gew.-%, vorteilhaft von mindestens 5, 6, 7, 8, 9 oder 10 Gew.-%, bevorzugt von mindestens 11, 12, 13, 14 oder 15 Gew.-%, besonders bevorzugt von mindestens 16, 17, 18, 19, oder 20 Gew.-%, ganz besonders bevorzugt von mindestens 25, 30, 35 oder 40 Gew.-% bezogen auf die gesamten Fettsäuren in der transgenen Pflanze hergestellt. Dabei sind die im erfindungsgemäßen Verfahren hergestellten Fettsäuren EPA, DPA und/oder DHA mit einem Gehalt von jeweils mindestens 5 Gew.-%, bevorzugt von jeweils mindestens 6, 7, 8 oder 9 Gew.-%, besonders bevorzugt von jeweils mindestens 10, 11 oder 12 Gew.-%, am meisten bevorzugt von jeweils mindestens 13, 14, 15, 16, 17, 18, 19 oder 20 Gew.-%, bezogen auf die gesamten Fettsäuren in der transgenen Pflanze enthalten.

Vorteilhaft werden die Fettsäuren in gebundener Form hergestellt. Mit Hilfe der im erfindungsgemäßen Verfahren verwendeten Nukleinsäuren lassen sich diese ungesättigten Fettsäuren an sn1-, sn2- und/oder sn3-Position der vorteilhaft hergestellten Triacylglyceride bringen. Vorteilhaft sind mindestens 11% der Triacylglyceride doppelt (das heißt an sn1- und sn2- oder sn2- und sn3-Position) substituiert. Auch dreifach substituierte Triacylglyceride sind nachweisbar. Da im erfindungsgemäßen Verfahren von den Ausgangsverbindungen Linolsäure (C18:2) bzw. Linolensäure (C18:3) mehrere Reaktionsschritte durchlaufen werden, fallen die Endprodukte des Verfahrens wie beispielsweise Arachidonsäure (ARA) oder Eicosapentaensäure (EPA) nicht als absolute Reinprodukte an, es sind immer auch Spuren oder größere Mengen der Vorstufen im Endprodukt enthalten. Sind in der Ausgangspflanze beispielsweise sowohl Linolsäure als auch Linolensäure vorhanden, so liegen die Endprodukte wie ARA oder EPA und/oder DPA und/oder DHA als Mischungen vor. Die Vorstufen sollten vorteilhaft nicht mehr als 20 Gew.-%, bevorzugt nicht mehr als 15 Gew.-%, besonders bevorzugt nicht als 10 Gew.-%, ganz besonders bevorzugt nicht mehr als 5 Gew.-% bezogen auf die Menge des jeweiligen Endprodukts betragen. Vorteilhaft werden in einer transgenen Pflanze als Endprodukte nur ARA oder EPA und/oder DPA und/oder DHA im erfindungsgemäßen Verfahren gebunden oder als freie Säuren hergestellt.

Fettsäureester bzw. Fettsäuregemische, die nach dem erfindungsgemäßen Verfahren hergestellt wurden, enthalten vorteilhaft 6 bis 15 % Palmitinsäure, 1 bis 6 % Stearinsäure; 7 – 85 % Ölsäure; 0,5 bis 8 % Vaccensäure, 0,1 bis 1 % Arachinsäure, 7 bis 25 % gesättigte Fettsäuren, 8 bis 85 % einfach ungesättigte Fettsäuren und 60 bis 85 % mehrfach ungesättigte Fettsäuren, jeweils bezogen auf 100 % und auf den Gesamtfettsäuregehalt der Organismen. Als vorteilhafte mehrfach ungesättigte Fettsäure sind in den Fettsäureester bzw. Fettsäuregemische bevorzugt mindestens 0,1; 0,2; 0,3; 0,4; 0,5; 0,6; 0,7; 0,8; 0,9 oder 1 % bezogen auf den Gesamtfettsäuregehalt an Arachidonsäure enthalten. Weiterhin enthalten die Fettsäureestern bzw. Fettsäuregemischen, die nach dem erfindungsgemäßen Verfahren hergestellt wurden, vorteilhaft Fettsäuren ausgewählt aus der Gruppe der Fettsäuren Erucasäure (13-Docosaensäure), Sterculinsäure (9,10-Methylene octadec-9-enonsäure), Malvalinsäure (8,9-Methylen Heptadec-8-enonsäure), Chaulmoogrinsäure (Cyclopenten-dodecansäure), Furan-Fettsäure (9,12-Epoxyoctadeca-9,11-dienonsäure), Vernonsäure (9,10-Epoxyoctadec-12-enonsäure), Tarinsäure (6-Octadecynonsäure),6-Nonadecynonsäure, Santalbinsäure (t11-Octadecen-9-ynoic acid), 6,9-Octadecenynonsäure, Pyrulinsäure (t10-Heptadecen-8-ynonsäure), Crepenyninsäure (9-Octadecen-12-ynonsäure), 13,14-Dihydrooropheinsäure, Octadecen-13-ene-9,11-diynonsäure, Petroselensäure (cis-6-Octadecenonsäure), 9c,12t-Octadecadiensäure, Calendulasäure (8t10t12c-Octadecatriensäure), Catalpinsäure (9t11t13c-Octadecatriensäure), Eleosterinsäure (9c11t13t-Octadecatriensäure), Jacarinsäure (8c10t12c-Octadecatriensäure), Punicinsäure (9c11t13c-Octadecatriensäure), Parinarinsäure (9c11t13t15c-Octadecatetraensäure), Pinolensäure (all-cis-5,9,12-Octadecatriensäure), Laballensäure (5,6-Octadecadienallensäure), Ricinolsäure (12-Hydroxyölsäure) und/oder Coriolinsäure (13-Hydroxy-9c,11t-Octadecadienonsäure). Die vorgenannten Fettsäuren kommen in den nach dem erfindungsgemäßen Verfahren hergestellten Fettsäureester bzw. Fettsäuregemischen in der Regel vorteilhaft nur in Spuren vor, das heißt sie kommen bezogen auf den Gesamtfettsäuregehalt zu weniger als 30 %, bevorzugt zu weniger als 25 %, 24 %, 23 %, 22 % oder 21 %, besonders bevorzugt zu weniger als 20 %, 15 %, 10 %, 9 %, 8 %, 7%, 6 % oder 5%, ganz besonders bevorzugt zu weniger als 4 %, 3 %, 2 % oder 1 % vor. In einer weiteren bevorzugten Form der Erfindung kommen diese vorgenannten Fettsäuren bezogen auf die Gesamtfettsäuren zu weniger als 0,9%; 0,8%; 0,7%; 0,6%; oder 0,5%, besonders bevorzugt zu weniger als

0,4%; 0,3%; 0,2%; 0,1% vor. Vorteilhaft enthalten die nach dem erfindungsgemäßen Verfahren hergestellten Fettsäureester bzw. Fettsäuregemische weniger als 0,1 % bezogen auf die Gesamtfettsäuren und/oder keine Buttersäure, kein Cholesterin sowie keine Nisinsäure (Tetracosahexaensäure, C23:6$^{\Delta 3,8,12,15,18,21}$).

Durch die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen kann in den transgenen Pflanzen eine Steigerung der Ausbeute an LCPUFAs von mindestens 50%, vorteilhaft von mindestens 80%, besonders vorteilhaft von mindestens 100%, ganz besonders vorteilhaft von mindestens 150% gegenüber den nicht transgenen Pflanzen erreicht werden.

Auch chemisch reine mehrfach ungesättigte Fettsäuren oder Fettsäurezusammensetzungen sind nach den vorbeschriebenen Verfahren darstellbar. Dazu werden die Fettsäuren oder die Fettsäurezusammensetzungen aus den Pflanzen in bekannter Weise beispielsweise über Extraktion, Destillation, Kristallisation, Chromatographie oder Kombinationen dieser Methoden isoliert. Diese chemisch reinen Fettsäuren oder Fettsäurezusammensetzungen sind für Anwendungen im Bereich der Lebensmittelindustrie, der Kosmetikindustrie und besonders der Pharmaindustrie vorteilhaft.

Für das erfindungsgemäße Verfahren sind prinzipiell alle dicotylen oder monokotylen Nutzpflanzen geeignet. Unter Nutzpflanzen sind Pflanzen zu verstehen, die der Nahrungsproduktion für Mensch und Tier, der Produktion von Genussmitteln, Fasern und Pharmazeutika dienen, wie Getreide, z.B. Mais, Reis, Weizen, Gerste, Hirse, Hafer, Roggen, Buchweizen; wie Knollen, z.B. Kartoffel, Maniok, Batate, Yams etc.; wie Zuckerpflanzen, z.B. Zuckerrohr oder Zuckerrübe; wie Hülsenfrüchte, z.B. Bohnen, Erbsen, Saubohne etc.; wie Öl- und Fettfrüchte, z.B. Sojabohne, Raps, Sonnenblume, Färberdiestel, Lein, Camelina etc., um nur einige zu nennen. Vorteilhafte Pflanzen sind ausgewählt aus der Gruppe der Pflanzenfamilien bestehend aus den Familien der Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalida-

ceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae und Valerianaceae.

Beispielhaft seien die folgenden Pflanzen genannt: Anacardiaceae wie die Gattungen Pistacia, Mangifera, Anacardium z.B. die Gattung und Arten Pistacia vera (Pistazie), Mangifer indica (Mango) oder Anacardium occidentale (Cashew), Asteraceae wie die Gattungen Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana z.B. die Gattung und Arten Calendula officinalis (Garten-Ringelblume), Carthamus tinctorius (Färberdistel, safflower), Centaurea cyanus (Kornblume), Cichorium intybus (Wegwarte), Cynara scolymus (Artischocke), Helianthus annus (Sonnenblume), Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta (Salat), Tagetes lucida, Tagetes erecta oder Tagetes tenuifolia (Studentenblume), Apiaceae wie die Gattung Daucus z.B. die Gattung und Art Daucus carota (Karotte), Betulaceae wie die Gattung Corylus z.B. die Gattungen und Arten Corylus avellana oder Corylus colurna (Haselnuss), Boraginaceae wie die Gattung Borago z.B. die Gattung und Art Borago officinalis (Borretsch), Brassicaceae wie die Gattungen Brassica, Camelina, Melanosinapis, Sinapis, Arabadopsis z.B. die Gattungen und Arten Brassica napus, Brassica rapa ssp. (Raps), Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Camelina sativa, Melanosinapis communis (Senf), Brassica oleracea (Futterrübe) oder Arabidopsis thaliana, Bromeliaceae wie die Gattungen Anana, Bromelia (Ananas) z.B. die Gattungen und Arten Anana comosus, Ananas ananas oder Bromelia comosa (Ananas), Caricaceae wie die Gattung Carica wie die Gattung und Art Carica papaya (Papaya), Cannabaceae wie die Gattung Cannabis wie die Gattung und Art Cannabis sative (Hanf), Convolvulaceae wie die Gattungen Ipomoea, Convolvulus z.B. die Gattungen und Arten Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba oder Convolvulus panduratus (Süßkartoffel, Batate), Chenopodiaceae wie die Gattung Beta wie die Gattungen und Arten Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var. vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva oder Beta vulgaris var. esculenta

(Zuckerrübe), Cucurbitaceae wie die Gattung Cucubita z.B. die Gattungen und Arten Cucurbita maxima, Cucurbita mixta, Cucurbita pepo oder Cucurbita moschata (Kürbis), Elaeagnaceae wie die Gattung Elaeagnus z.B. die Gattung und Art Olea europaea (Olive), Ericaceae wie die Gattung Kalmia z.B. die Gattungen und Arten Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros oder Kalmia lucida (Berglorbeer), Euphorbiaceae wie die Gattungen Manihot, Janipha, Jatropha, Ricinus z.B. die Gattungen und Arten Manihot utilissima, Janipha manihot,, Jatropha manihot., Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta (Manihot) oder Ricinus communis (Rizinus), Fabaceae wie die Gattungen Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja z.B. die Gattungen und Arten Pisum sativum, Pisum arvense, Pisum humile (Erbse), Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa (Seidenbaum), Medicago sativa, Medicago falcata, Medicago varia (Alfalfa) Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida oder Soja max (Sojabohne), Geraniaceae wie die Gattungen Pelargonium, Cocos, Oleum z.B. die Gattungen und Arten Cocos nucifera, Pelargonium grossularioides oder Oleum cocois (Kokusnuss), Gramineae wie die Gattung Saccharum z.B. die Gattung und Art Saccharum officinarum, Juglandaceae wie die Gattungen Juglans, Wallia z.B. die Gattungen und Arten Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra oder Wallia nigra (Walnuss), Lauraceae wie die Gattungen Persea, Laurus z.B. die Gattungen und Arten Laurus nobilis (Lorbeer), Persea americana, Persea gratissima oder Persea persea (Avocado), Leguminosae wie die Gattung Arachis z.B. die Gattung und Art Arachis hypogaea (Erdnuss), Linaceae wie die Gattungen Linum, Adenolinum z.B. die Gattungen und Arten Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Li-

num catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense oder Linum trigynum (Lein), Lythrarieae wie die Gattung Punica z.B. die Gattung und Art Punica granatum (Granatapfel), Malvaceae wie die Gattung Gossypium z.B. die Gattungen und Arten Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum oder Gossypium thurberi (Baumwolle), Musaceae wie die Gattung Musa z.B. die Gattungen und Arten Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. (Banane), Onagraceae wie die Gattungen Camissonia, Oenothera z.B. die Gattungen und Arten Oenothera biennis oder Camissonia brevipes (Nachtkerze), Palmae wie die Gattung Elaeis z.B. die Gattung und Art Elaeis guineensis (Ölpalme), Papaveraceae wie die Gattung Papaver z.B. die Gattungen und Arten Papaver orientale, Papaver rhoeas, Papaver dubium (Mohn), Pedaliaceae wie die Gattung Sesamum z.B. die Gattung und Art Sesamum indicum (Sesam), Piperaceae wie die Gattungen Piper, Artanthe, Peperomia, Steffensia z.B. die Gattungen und Arten Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata (Cayennepfeffer), Poaceae wie die Gattungen Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (Mais), Triticum, z.B. die Gattungen und Arten Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon Hordeum aegiceras, Hordeum hexastichon., Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum (Gerste), Secale cereale (Roggen), Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida (Hafer), Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum (Hirse), Oryza sativa, Oryza latifolia (Reis), Zea mays (Mais) Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum oder Triticum vulgare (Weizen), Porphyridiaceae wie die Gattungen Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Van-

hoeffenia z.B. die Gattung und Art Porphyridium cruentum, Proteaceae wie die Gattung Macadamia z.B. die Gattung und Art Macadamia intergrifolia (Macadamia), Rubiaceae wie die Gattung Coffea z.B. die Gattungen und Arten Cofea spp., Coffea arabica, Coffea canephora oder Coffea liberica (Kaffee), Scrophulariaceae wie die Gattung Verbascum z.B. die Gattungen und Arten Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum oder Verbascum thapsus (Königskerze), Solanaceae wie die Gattungen Capsicum, Nicotiana, Solanum, Lycopersicon z.B. die Gattungen und Arten Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens (Pfeffer), Capsicum annuum (Paprika), Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris (Tabak), Solanum tuberosum (Kartoffel), Solanum melongena (Aubergine) Lycopersicon esculentum, Lycopersicon lycopersicum., Lycopersicon pyriforme, Solanum integrifolium oder Solanum lycopersicum (Tomate), Sterculiaceae wie die Gattung Theobroma z.B. die Gattung und Art Theobroma cacao (Kakao) oder Theaceae wie die Gattung Camellia z.B. die Gattung und Art Camellia sinensis (Tee).

In einer vorteilhaften Ausführungsform des Verfahren werden als Nutzpflanzen Ölfruchtpflanzen verwendet, die große Mengen an Lipidverbindungen enthalten, wie Erdnuss, Raps, Canola, Sonnenblume, Saflor (Carthamus tinctoria), Mohn, Senf, Hanf, Rizinus, Olive, Sesam, Calendula, Punica, Nachtkerze, Königskerze, Distel, Wildrosen, Haselnuss, Mandel, Macadamia, Avocado, Lorbeer, Kürbis, Lein, Soja, Pistazien, Borretsch, Bäume (Ölpalme, Kokosnuss oder Walnuss) oder Feldfrüchte, wie Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Baumwolle, Maniok, Pfeffer, Tagetes, Solanaceen-Pflanzen wie Kartoffel, Tabak, Aubergine und Tomate, Vicia-Arten, Erbse, Alfalfa oder Buschpflanzen (Kaffee, Kakao, Tee), Salix-Arten sowie ausdauernde Gräser und Futterfeldfrüchte. Vorteilhafte erfindungsgemäße Pflanzen sind Ölfruchtpflanzen, wie Erdnuss, Raps, Canola, Sonnenblume, Saflor , Mohn, Senf, Hanf, Rhizinus, Olive, Calendula, Punica, Nachtkerze, Kürbis, Lein, Soja, Borretsch, Bäume (Ölpalme, Kokosnuss). Besonders bevorzugt sind Pflanzen, die reich an C18:2- und/oder C18:3-Fettsäuren sind, wie

Sonnenblume, Färberdistel, Tabak, Königskerze, Sesam, Baumwolle, Kürbis, Mohn, Nachtkerze, Walnuss, Lein, Hanf oder Distel. Ganz besonders bevorzugt sind Pflanzen wie Färberdistel, Sonnenblume, Mohn, Nachtkerze, Walnuss, Lein oder Hanf.

Es ist auch vorteilhaft, die erfindungsgemäßen Nukleinsäuresequenzen in den Blättern von Futter- oder Nahrungspflanzen zu exprimieren und dadurch den Gehalt der Blätter an Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure zu steigern. Bevorzugte Futterpflanzen sind z.B. Kleearten wie Rotklee (Trifolium pratense), Weißklee (Trifolium repens), Bastardklee (Trifolium hybridum), Esparsette (Onobrychis viciifolia), Alexandrinerklee (Trifolium alexandrinum) und Perserklee (Trifolium resupinatum). Bevorzugte Nahrungspflanzen sind etwa Salatarten wie Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis und Valeriana locusta.

Durch die enzymatische Aktivität der im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen, die für Polypeptide mit Δ-6-Elongase-, Δ-6-Desaturase-, Δ-5-Desaturase- und/oder Δ-5-Elongase-Aktivität kodieren, vorteilhaft in Kombination mit Nukleinsäuresequenzen, die für Polypeptide mit ω-3-Desaturase- und/oder Δ-4-Desaturase-Aktivität kodieren, sowie weiteren Nukleinsäuresequenzen, die für Polypeptide des Fettsäure– oder Lipidstoffwechsels wie weiteren Polypeptiden mit Δ-5-, Δ–6–, Δ-8-, Δ-12-Desaturase- oder Δ-5-, Δ–6–oder Δ-9-Elongaseaktivität kodieren, können unterschiedlichste mehrfach ungesättigte Fettsäuren im erfindungsgemäßen Verfahren hergestellt werden. Je nach Auswahl der für das erfindungsgemäße Verfahren verwendeten Nutzpflanzen lassen sich Mischungen der verschiedenen mehrfach ungesättigten Fettsäuren oder einzelne mehrfach ungesättigte Fettsäuren wie EPA, DPA oder DHA in freier oder gebundener Form herstellen. Je nachdem welche Fettsäurezusammensetzung in der Ausgangspflanze vorherrscht (C18:2- oder C18:3-Fettsäuren) entstehen so Fettsäuren, die sich von C18:2-Fettsäuren ableiten, wie GLA, DGLA oder ARA oder Fettsäuren, die sich von C18:3-Fettsäuren ableiten, wie EPA, DPA oder DHA. Liegt in der für das Verfahren verwendeten Pflanze als ungesättigte Fettsäure nur Linolsäure (= LA, $C18:2^{\Delta 9,12}$) vor, so können als Produkte des Verfahrens nur GLA, DGLA und ARA ent-

stehen, die als freie Fettsäuren oder gebunden vorliegen können. Ist in der im Verfahren verwendeten Pflanze als ungesättigte Fettsäure nur α-Linolensäure (= ALA, C18:3$^{\Delta 9,12,15}$)vorhanden, beispielsweise wie in Lein, so können als Produkte des Verfahrens nur SDA, ETA, EPA, DPA und/oder DHA entstehen, die wie oben beschrieben als freie Fettsäuren oder gebunden vorliegen können. Durch Modifikation der Aktivität der im Verfahren verwendeten und an der Synthese beteiligten Enzyme Δ-6-Elongase, Δ-6-Desaturase, Δ-5-Desaturase und/oder Δ-6-Elongase vorteilhaft in Kombination mit weiteren Genen des Lipid- oder Fettsäurestoffwechsels lassen sich gezielt in den Pflanzen nur einzelne Produkte herstellen. Vorteilhaft werden nur EPA, DPA oder DHA oder deren Mischungen synthetisiert. Da die Fettsäuren in Biosyntheseketten synthetisiert werden, liegen die jeweiligen Endprodukte nicht als Reinsubstanzen in den Organismen vor. Es sind immer auch geringe Mengen der Vorläuferverbindungen im Endprodukt enthalten. Diese geringen Mengen betragen weniger als 20 Gew.-%, vorteilhaft weniger als 15 Gew.-%, besonders vorteilhaft weniger als 10 Gew.-%, ganz besonders vorteilhaft weniger als 5, 4, 3, 2 oder 1 Gew.-% bezogen auf die Endprodukte EPA, DPA oder DHA oder deren Mischungen.

Zur Steigerung der Ausbeute im beschriebenen Verfahren zur Herstellung von Ölen und/oder Triglyceriden mit einem vorteilhaft erhöhten Gehalt an mehrfach ungesättigten Fettsäuren ist es vorteilhaft, die Menge an Ausgangsprodukt für die Fettsäuresynthese zu steigern. Dies kann beispielsweise durch das Einbringen einer Nukleinsäure, die für ein Polypeptid mit Δ-12-Desaturase kodiert, in den Organismus erreicht werden. Dies ist besonders vorteilhaft in Nutzpflanzen, wie Öl-produzierenden Pflanzen wie Pflanzen der Familie der Brassicaceae wie der Gattung Brassica z.B. Raps; der Familie der Elaeagnaceae wie die Gattung Elaeagnus z.B. die Gattung und Art Olea europaea oder der Familie Fabaceae wie der Gattung Glycine z.B. die Gattung und Art Glycine max, die einen hohen Ölsäuregehalt aufweisen. Da diese Organismen nur einen geringen Gehalt an Linolsäure aufweisen (Mikoklajczak et al. (1961) Journal of the American Oil Chemical Society 38: 678 - 681) ist die Verwendung der genannten Δ-12-Desaturasen zur Herstellung des Ausgangsprodukts Linolsäure aus Ölsäure vorteilhaft. Daneben können die Ausgangsfettsäuren auch von außen zugefüttert werden, was aber aus Kostengründen weniger bevorzugt ist.

Moose und Algen sind die einzigen bekannten Pflanzensysteme, die erhebliche Mengen an mehrfach ungesättigten Fettsäuren, wie Arachidonsäure (ARA) und/oder Eicosapentaensäure (EPA) und/oder Docosahexaensäure (DHA) herstellen. Moose enthalten PUFAs in Membranlipiden, während Algen, algenverwandte Organismen und einige Pilze auch nennenswerte Mengen an PUFAs in der Triacylglycerolfraktion akkumulieren. Daher eignen sich Nukleinsäuremoleküle, die aus solchen Stämmen isoliert werden, die PUFAs auch in der Triacylglycerolfraktion akkumulieren, besonders vorteilhaft für das erfindungsgemäße Verfahren und damit zur Modifikation des Lipid- und PUFA-Produktionssystems in einer Pflanze, wie einer Nutzpflanze wie einer Ölfruchtpflanze, beispielsweise Raps, Canola, Lein, Hanf, Soja, Sonnenblumen, Borretsch. Sie sind deshalb vorteilhaft im erfindungsgemäßen Verfahren verwendbar.

Im erfindungsgemäßen Verfahren verwendete Nukleinsäuren stammen vorteilhaft aus Pflanzen wie Algen, beispielsweise Algen der Familie der Prasinophyceae wie aus den Gattungen Heteromastix, Mammella, Mantoniella, Micromonas, Nephroselmis, Ostreococcus, Prasinocladus, Prasinococcus, Pseudoscourfielda, Pycnococcus, Pyramimonas, Scherffelia oder Tetraselmis wie den Gattungen und Arten Heteromastix longifillis, Mamiella gilva, Mantoniella squamata, Micromonas pusilla, Nephroselmis olivacea, Nephroselmis pyriformis, Nephroselmis rotunda, Ostreococcus tauri, Ostreococcus sp. Prasinocladus ascus, Prasinocladus lubricus, Pycnococcus provasolii, Pyramimonas amylifera, Pyramimonas disomata, Pyramimonas obovata, Pyramimonas orientalis, Pyramimonas parkeae, Pyramimonas spinifera, Pyramimonas sp., Tetraselmis apiculata, Tetraselmis carteriaformis, Tetraselmis chui, Tetraselmis convolutae, Tetraselmis desikacharyi, Tetraselmis gracilis, Tetraselmis hazeni, Tetraselmis impellucida, Tetraselmis inconspicua, Tetraselmis levis, Tetraselmis maculata, Tetraselmis marina, Tetraselmis striata, Tetraselmis subcordiformis, Tetraselmis suecica, Tetraselmis tetrabrachia, Tetraselmis tetrathele, Tetraselmis verrucosa, Tetraselmis verrucosa fo. rubens oder Tetraselmis sp. oder aus Algen der Familie Euglenaceae wie aus den Gattungen Ascoglena, Astasia, Colacium, Cyclidiopsis, Euglena, Euglenopsis, Hyalophacus, Khawkinea, Lepocinclis, Phacus, Strombomonas oder Trachelomonas wie die Gattungen und Art Euglena acus, Euglena geniculata, Euglena gracilis, Euglena mixocylindracea, Euglena rostrifera,

Euglena viridis, Colacium stentorium, Trachelomonas cylindrica oder Trachelomonas volvocina.

Weitere vorteilhafte Pflanzen sind Algen wie Isochrysis oder Crypthecodinium, Algen/Diatomeen wie Thalassiosira oder Phaeodactylum, Moose wie Physcomitrella oder Ceratodon oder höheren Pflanzen wie den Primulaceae wie Aleuritia, Calendula stellata, Osteospermum spinescens oder Osteospermum hyoseroides, Mikroorganismen wie Pilzen wie Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor oder Mortierella, Bakterien wie Shewanella, Hefen oder Tiere wie Nematoden wie Caenorhabditis, Insekten, Frösche, Seegurken oder Fische. Vorteilhaft stammen die erfindungsgemäßen isolierten Nukleinsäuresequenzen aus einem Tier aus der Ordnung der Vertebraten. Bevorzugt stammen die Nukleinsäuresequenzen aus der Klasse der Vertebrata; Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae bzw. Oncorhynchus oder Vertebrata, Amphibia, Anura, Pipidae, Xenopus oder Evertebrata wie Protochordata, Tunicata, Holothuroidea, Cionidae wie Amaroucium constellatum, Botryllus schlosseri, Ciona intestinalis, Molgula citrina, Molgula manhattensis, Perophora viridis oder Styela partita. Besonders vorteilhaft stammen die Nukleinsäuren aus Pilzen, Tieren oder aus Pflanzen wie Algen oder Moosen, bevorzugt aus der Ordnung der Salmoniformes wie der Familie der Salmonidae wie der Gattung Salmo beispielsweise aus den Gattungen und Arten Oncorhynchus mykiss, Trutta trutta oder Salmo trutta fario, aus Algen wie den Gattungen Mantoniella oder Ostreococcus oder aus den Diatomeen wie den Gattungen Thalassiosira oder Phaeodactylum oder aus Algen wie Crypthecodinium.

Bei einer bevorzugten Ausführungsform umfasst das Verfahren ferner den Schritt des Gewinnens einer Zelle oder einer ganzen Pflanze, die die im Verfahren verwendeten Nukleinsäuresequenzen, die für eine Δ-6-Desaturase, Δ-6-Elongase, Δ-5-Desaturase und/oder Δ-5-Elongase sowie ggf. Nukleinsäuresequenzen, die für eine ω-3-Desaturase und/oder eine Δ-4-Desaturase kodieren, enthält, wobei die Zelle und/oder die Nutzpflanze noch weitere Nukleinsäuresequenzen des Lipid- oder Fettsäurestoffwechsels enthalten kann. Die im Verfahren bevorzugt verwendeten Nukleinsäuresequenzen werden zur Expression vorteilhaft in mindestens ein Genkonstrukt und/oder einen Vektor wie nach-

folgend beschrieben, allein oder in Kombination mit weiteren Nukleinsäuresequenzen, die für Proteine des Fettsäure- oder Lipidstoffwechsels kodieren, eingebaut und schließlich in die Zelle oder Pflanze transformiert. Bei einer weiteren bevorzugten Ausführungsform umfasst dieses Verfahren ferner den Schritt des Gewinnens der Öle, Lipide oder freien Fettsäuren aus den Nutzpflanzen. Die so hergestellte Zelle oder die so hergestellte Nutzpflanze ist vorteilhaft eine Zelle einer Öl-produzierenden Pflanze, Gemüse-, Salat-, oder Zierpflanze oder die Pflanze selbst wie oben ausgeführt.

Unter Anzucht ist beispielsweise die Kultivierung im Falle von Pflanzenzellen, -gewebe oder -organe auf oder in einem Nährmedium oder der ganzen Pflanze auf bzw. in einem Substrat beispielsweise in Hydrokultur, Blumentopferde oder auf einem Ackerboden zu verstehen.

"Transgen" bzw. "rekombinant" im Sinne der Erfindung bedeutet bezüglich zum Beispiel einer Nukleinsäuresequenz, einer Expressionskassette (= Genkonstrukt) oder einem Vektor enthaltend die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen oder einer mit den im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen, Expressionskassette oder Vektor transformierten Pflanze alle solche durch gentechnische Methoden zustandegekommenen Konstruktionen, in denen sich entweder

a) die Nukleinsäuresequenz, oder

b) eine mit der Nukleinsäuresequenz funktionell verknüpfte genetische Kontrollsequenz, zum Beispiel ein Promotor, oder

c) (a) und (b)

nicht in ihrer natürlichen, genetischen Umgebung befinden oder durch gentechnische Methoden modifiziert wurden, wobei die Modifikation beispielhaft eine Substitution, Addition, Deletion, Inversion oder Insertion eines oder mehrerer Nukleotidreste sein kann. Natürliche genetische Umgebung meint den natürlichen genomischen bzw. chromosomalen Locus in dem Herkunftsorganismus oder das Vorliegen in einer genomischen Bibliothek. Im Fall einer genomischen Bibliothek ist die natürliche, genetische Umgebung

der Nukleinsäuresequenz bevorzugt zumindest noch teilweise erhalten. Die Umgebung flankiert die Nukleinsäuresequenz zumindest an einer Seite und hat eine Sequenzlänge von mindestens 50 bp, bevorzugt mindestens 500 bp, besonders bevorzugt mindestens 1000 bp, ganz besonders bevorzugt mindestens 5000 bp. Eine natürlich vorkommende Expressionskassette - beispielsweise die natürlich vorkommende Kombination des natürlichen Promotors der im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenz mit der Nukleinsäuresequenz, die für Proteine mit entsprechender Δ-6-Desaturase-, Δ-6-Elongase-, Δ-5-Desaturase- und Δ-5-Elongase-Aktivität kodiert, vorteilhaft in Kombination mit Nukleinsäuresequenzen, die für Proteine mit ω-3-Desaturase- und/oder Δ-4-Desaturase-Aktivität kodieren - wird zu einer transgenen Expressionskassette, wenn diese durch nicht-natürliche, synthetische ("künstliche") Verfahren wie beispielsweise einer Mutagenisierung geändert wird. Entsprechende Verfahren sind beispielsweise beschrieben in US 5,565,350 oder WO 00/15815.

Unter "transgener Pflanze" im Sinne der Erfindung ist wie vorgenannt zu verstehen, dass die im Verfahren verwendeten Nukleinsäuren nicht an ihrer natürlichen Stelle im Genom der Pflanze sind. Dabei können die Nukleinsäuresequenzen homolog oder heterolog exprimiert werden. Transgen bedeutet aber auch, dass die erfindungsgemäßen Nukleinsäuren an ihrem natürlichen Platz im Genom der Pflanze sind, dass jedoch die Sequenz gegenüber der natürlichen Sequenz verändert wurde und/oder dass die Regulationssequenzen der natürlichen Sequenzen verändert wurden. Bevorzugt ist unter transgen die Expression der im erfindungsgemäßen Verfahren verwendeten Nukleinsäuren an nicht-natürlicher Stelle im Genom zu verstehen, das heißt eine homologe oder bevorzugt heterologe Expression der Nukleinsäuresequenzen liegt vor.

Bevorzugte transgene Organismen sind Nutzpflanzen wie Öl-produzierende Pflanzen, Gemüse-, Salat- oder Zierpflanzen, die vorteilhaft ausgewählt sind aus der Gruppe der Pflanzenfamilien bestehend aus den Familien der Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Li-

naceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae und Valerianaceae.

Als Wirtspflanzen für die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuren, die Expressionskassette oder den Vektor eignen sich prinzipiell vorteilhaft alle Nutzpflanzen, die in der Lage sind Fettsäuren, speziell ungesättigte Fettsäuren, zu synthetisieren bzw. die für die Expression rekombinanter Gene geeignet sind. Beispielhaft seien an dieser Stelle Pflanzen wie Arabidopsis, Asteraceae wie Calendula oder Nutzpflanzen wie Soja, Erdnuss, Rizinus, Sonnenblume, Mais, Baumwolle, Flachs, Raps, Kokosnuss, Ölpalme, FärberSaflor (Carthamus tinctorius) oder Kakaobohne genannt. Weitere vorteilhafte Pflanzen sind an anderer Stelle dieser Anmeldung aufgeführt.

Für die Herstellung der transgenen Nutzpflanze werden in der Regel als Zwischenwirte Mikroorganismen verwendet. Derartige nutzbare Zwischenwirtszellen werden in: Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) genannt.

Vorteilhaft verwendbare Expressionsstämme für diesen Zweck sind z.B. solche, die eine geringe Proteaseaktivität aufweisen. Sie werden z.B. in: Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128 beschrieben.

Transgene Pflanzen, die die im erfindungsgemäßen Verfahren synthetisierten mehrfach ungesättigten, langekettigen Fettsäuren enthalten, können vorteilhaft direkt vermarktet werden, ohne dass die synthetisierten Öle, Lipide oder Fettsäuren isoliert werden müssen. Diese Form der Vermarktung ist besonders vorteilhaft.

Bei den "Pflanzen" im Sinne der vorliegenden Erfindung handelt es sich um ganze Pflanzen sowie alle Pflanzenteile, Pflanzenorgane oder Pflanzenteile wie Blatt, Stiel, Samen, Wurzel, Knollen, Antheren, Fasern, Wurzelhaare, Stängel, Embryos, Kalli, Kotelydonen, Petiolen, Erntematerial, pflanzliches Gewebe, reproduktives Gewebe, Zellkulturen, die

sich von der transgenen Pflanze ableiten und/oder dazu verwendet werden können, die transgene Pflanze hervorzubringen. Der Samen umfasst dabei alle Samenteile wie die Samenhüllen, Epidermis- und Samenzellen, Endosperm oder Embyrogewebe.

Die im erfindungsgemäßen Verfahren hergestellten Verbindungen können aber auch aus den Pflanzen in Form ihrer Öle, Fett, Lipide und/oder freien Fettsäuren isoliert werden. Durch das erfindungsgemäße Verfahren hergestellte mehrfach ungesättigte Fettsäuren lassen sich durch Ernten der Pflanzen oder Pflanzenzellen entweder aus der Kultur, in der sie wachsen, oder vom Feld gewinnen. Dies kann über Pressen oder Extraktion der Pflanzenteile, bevorzugt der Pflanzensamen, erfolgen. Dabei können die Öle, Fette, Lipide und/oder freien Fettsäuren durch so genanntes Kaltschlagen oder Kaltpressen ohne Zuführung von Wärme durch Pressen gewonnen werden. Damit sich die Pflanzenteile, speziell die Samen, leichter aufschließen lassen, werden sie vorher zerkleinert, gedämpft oder geröstet. Die so vorbehandelten Samen können anschließend gepresst oder mit Lösungsmittel wie warmem Hexan extrahiert werden. Anschließend wird das Lösungsmittel wieder entfernt. Auf diese Weise können mehr als 96 % der im erfindungsgemäßen Verfahren hergestellten Verbindungen isoliert werden. Anschließend werden die so erhaltenen Produkte weiter bearbeitet, das heißt raffiniert. Dabei werden zunächst beispielsweise die Pflanzenschleime und Trübstoffe entfernt. Die sogenannte Entschleimung kann enzymatisch oder beispielsweise chemisch/physikalisch durch Zugabe von Säure wie Phosphorsäure erfolgen. Anschließend werden die freien Fettsäuren durch Behandlung mit einer Base beispielsweise Natronlauge entfernt. Das erhaltene Produkt wird zur Entfernung der im Produkt verbliebenen Lauge mit Wasser gründlich gewaschen und getrocknet. Um die noch im Produkt enthaltenen Farbstoffe zu entfernen, werden die Produkte einer Bleichung mit beispielsweise Bleicherde oder Aktivkohle unterzogen. Zum Schluss wird das Produkt noch beispielsweise mit Wasserdampf desodoriert.

Vorzugsweise sind die durch dieses Verfahren produzierten PUFAs bzw. LCPUFAs C_{20}- und/oder C_{22}-Fettsäuremoleküle mit mindestens vier Doppelbindungen im Fettsäuremolekül, vorzugsweise fünf oder sechs Doppelbindungen. Diese C_{20}- und/oder C_{22}-Fettsäuremoleküle lassen sich aus der Pflanze in Form eines Öls, Lipids oder einer frei-

en Fettsäure isolieren. Geeignete transgene Pflanzen sind beispielsweise die vorstehend erwähnten.

Diese erfindungsgemäßen Öle, Lipide oder Fettsäuren enthalten wie oben beschrieben vorteilhaft 6 bis 15 % Palmitinsäure, 1 bis 6 % Stearinsäure; 7 – 85 % Ölsäure; 0,5 bis 8 % Vaccensäure, 0,1 bis 1 % Arachinsäure, 7 bis 25 % gesättigte Fettsäuren, 8 bis 85 % einfach ungesättigte Fettsäuren und 60 bis 85 % mehrfach ungesättigte Fettsäuren jeweils bezogen auf 100 % und auf den Gesamtfettsäuregehalt der Pflanzen.

Als vorteilhafte mehrfach ungesättigte, langkettige Fettsäuren sind in den Fettsäureestern bzw. Fettsäuregemischen wie Phosphatidylfettsäureestern oder Triacylglyceridestern bevorzugt mindestens 10; 11; 12; 13; 14; 15; 16; 17; 18; 19 oder 20 Gew.-% bezogen auf den Gesamtfettsäuregehalt an Eicosapentaensäure und/oder mindestens 1; 2; 3; 4; 5 oder 6 Gew.-% bezogen auf den Gesamtfettsäuregehalt an Docosapentaensäure und/oder mindestens 1; 2; 3; bevorzugt mindestens 4; 5; 6; besonders bevorzugt mindestens 7 oder 8 und am meisten bevorzugt mindestens 9 oder 10 Gew.-% bezogen auf den Gesamtfettsäuregehalt an Docosahexaensäure enthalten.

Weiterhin enthalten die Fettsäureester bzw. Fettsäuregemische, die nach dem erfindungsgemäßen Verfahren hergestellt wurden, Fettsäuren ausgewählt aus der Gruppe der Fettsäuren Erucasäure (13-Docosaensäure), Sterculinsäure (9,10-Methylene octadec-9-enonsäure), Malvalinsäure (8,9-Methylen Heptadec-8-enonsäure), Chaulmoogrinsäure (Cyclopentendodecansäure), Furan-Fettsäure (9,12-Epoxy-octadeca-9,11-dienonsäure), Vernonsäure (9,10-Epoxyoctadec-12-enonsäure), Tarinsäure (6-Octadecynonsäure),6-Nonadecynonsäure, Santalbinsäure (t11-Octadecen-9-ynoic acid), 6,9-Octadecenynonsäure, Pyrulinsäure (t10-Heptadecen-8-ynonsäure), Crepenyninsäure (9-Octadecen-12-ynonsäure), 13,14-Dihydrooropheinsäure, Octadecen-13-ene-9,11-diynonsäure, Petroselensäure (cis-6-Octadecenonsäure), 9c,12t-Octadecadiensäure, Calendulasäure (8t10t12c-Octadecatriensäure), Catalpinsäure (9t11t13c-Octadecatriensäure), Eleosterinsäure (9c11t13t-Octadecatriensäure), Jacarinsäure (8c10t12c-Octadecatriensäure), Punicinsäure (9c11t13c-Octadecatriensäure), Parinarinsäure (9c11t13t15c-Octadecatetraensäure), Pinolensäure (all-cis-5,9,12-

Octadecatriensäure), Laballensäure (5,6-Octadecadienallensäure), Ricinolsäure (12-Hydroxyölsäure) und/oder Coriolinsäure (13-Hydroxy-9c,11t-Octadecadienonsäure). Die vorgenannten Fettsäuren kommen in den nach dem erfindungsgemäßen Verfahren hergestellten Fettsäureester bzw. Fettsäuregemischen in der Regel vorteilhaft nur in Spuren vor, das heißt sie kommen bezogen auf die Gesamtfettsäuren zu weniger als 30 %, bevorzugt zu weniger als 25 %, 24 %, 23 %, 22 % oder 21 %, besonders bevorzugt zu weniger als 20 %, 15 %, 10 %, 9 %, 8 %, 7%, 6 % oder 5%, ganz besonders bevorzugt zu weniger als 4 %, 3 %, 2 % oder 1 % vor. In einer weiteren bevorzugten Form der Erfindung kommen diese vorgenannten Fettsäuren bezogen auf die Gesamtfettsäuren zu weniger als 0,9%; 0,8%; 0,7%; 0,6%; oder 0,5%, besonders bevorzugt zu weniger als 0,4%; 0,3%; 0,2%; 0,1% vor. Vorteilhaft enthalten die nach dem erfindungsgemäßen Verfahren hergestellten Fettsäureester bzw. Fettsäuregemische weniger als 0,1 % bezogen auf die Gesamtfettsäuren und/oder keine Buttersäure, kein Cholesterin sowie keine Nisinsäure.

Eine weitere erfindungsgemäße Ausführungsform ist die Verwendung der Öle, Lipide, der Fettsäuren und/oder der Fettsäurezusammensetzung, die nach dem erfindungsgemäßen Verfahren hergestellt werden, in Futtermitteln, Nahrungsmitteln, Kosmetika oder Pharmazeutika. Die im erfindungsgemäßen Verfahren gewonnenen Öle, Lipide, Fettsäuren oder Fettsäuregemische können in der dem Fachmann bekannten Weise zur Abmischung mit anderen Ölen, Lipiden, Fettsäuren oder Fettsäuregemischen tierischen Ursprungs wie z.B. Fischölen verwendet werden. Auch diese so hergestellten Öle, Lipide, Fettsäuren oder Fettsäuregemische, die aus pflanzlichen und tierischen Bestandteilen bestehen, können zur Herstellung von Futtermitteln, Nahrungsmitteln, Kosmetika oder Pharmazeutika verwendet werden.

Unter dem Begriff “Öl”, “Lipid“ oder “Fett” wird ein Fettsäuregemisch verstanden, das ungesättigte und/oder gesättigte, vorzugsweise veresterte Fettsäure(n) enthält. Bevorzugt ist, dass das Öl, Lipid oder Fett einen hohen Anteil an mehrfach ungesättigten freien oder vorteilhaft veresterten Fettsäure(n), insbesondere Linolsäure, γ-Linolensäure, Dihomo-γ-linolensäure, Arachidonsäure, α-Linolensäure, Stearidonsäure, Eicosatetraensäure, Eicosapentaensäure, Docosapentaensäure oder Docosahexaensäure hat. Vor-

zugsweise ist der Anteil an ungesättigten veresterten Fettsäuren ungefähr 30 %, besonders bevorzugt ist ein Anteil von 50 %, am meisten bevorzugt ist ein Anteil von 60 %, 70 %, 80 % oder mehr. Der Anteil an Fettsäure kann nach Überführung der Fettsäuren in die Methylester durch Umesterung gaschromatographisch bestimmt werden. Das Öl, Lipid oder Fett kann verschiedene andere gesättigte oder ungesättigte Fettsäuren, z.B. Calendulasäure, Palmitin-, Palmitolein-, Stearin-, Ölsäure etc., enthalten. Insbesondere kann je nach Ausgangspflanze der Anteil der verschiedenen Fettsäuren in dem Öl oder Fett schwanken.

Bei den im Verfahren hergestellten mehrfach ungesättigte Fettsäuren mit vorteilhaft mindestens drei, vier, fünf oder sechs, besonders vorteilhaft mit fünf oder sechs Doppelbindungen, handelt es sich wie oben beschrieben vorteilhaft um Fettsäureester beispielsweise um Sphingolipidester, Phosphoglyceridester, Lipidester, Glycolipidester, Phospholipidester, Monoacylglycerinester, Diacylglycerinester, Triacylglycerinester oder sonstige Fettsäureester, bevorzugt handelt es sich um Phospholipidester und/oder Triacylglycerinester.

Aus den so im erfindungsgemäßen Verfahren hergestellten mehrfach ungesättigte Fettsäureestern mit vorteilhaft mindestens drei, vier, fünf oder sechs Doppelbindungen lassen sich die enthaltenen mehrfach ungesättigten Fettsäuren beispielsweise über eine Alkalibehandlung, beispielsweise mit wäßriger KOH oder NaOH, oder durch saure Hydrolyse vorteilhaft in Gegenwart eines Alkohols wie Methanol oder Ethanol oder über eine enzymatische Abspaltung freisetzen und isolieren über beispielsweise Phasentrennung und anschließende Ansäuerung mit z.B. H_2SO_4. Die Freisetzung der Fettsäuren kann auch direkt ohne die vorhergehend beschriebene Aufarbeitung erfolgen.

Als Substrate der im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen, die für Polypeptide mit Δ-6-Desaturase-, Δ-6-Elongase-, Δ-5-Desaturase- und/oder Δ-5-Elongase-Aktivität sowie ggf. Nukleinsäuresequenzen, die für Polypeptide mit ω-3-Desaturase- und/oder Δ-4-Desaturase-Aktivität kodieren, und/oder den weiteren verwendeten Nukleinsäuren wie den Nukleinsäuresequenzen, die für Polypeptide des Fettsäure– oder Lipidstoffwechsels ausgewählt aus der Gruppe Acyl-CoA-

Dehydrogenase(n), Acyl-ACP(= acyl carrier protein)–Desaturase(n), Acyl–ACP–Thioesterase(n), Fettsäure–Acyl–Transferase(n), Acyl-CoA:Lysophospholipid-Acyltransferase(n), Fettsäure–Synthase(n), Fettsäure–Hydroxylase(n), Acetyl-Coenzym A–Carboxylase(n), Acyl–Coenzym A–Oxidase(n), Fettsäure–Desaturase(n), Fettsäure–Acetylenase(n), Lipoxygenase(n), Triacylglycerol–Lipase(n), Allenoxid–Synthase(n), Hydroperoxid–Lyase(n) oder Fettsäure–Elongase(n) kodieren, eignen sich vorteilhaft C_{16}-, C_{18}- oder C_{20}-Fettsäuren. Bevorzugt werden die im Verfahren als Substrate umgesetzten Fettsäuren in Form ihrer Acyl-CoA-Ester und/oder ihrer Phospholipid-Ester umgesetzt.

Zur Herstellung der erfindungsgemäßen langkettigen PUFAs müssen die gesättigten, einfach ungesättigten C_{16}-Fettsäuren und/oder mehrfach ungesättigten C_{18}-Fettsäuren zunächst je nach Substrat durch die enzymatische Aktivität einer Desaturase und/oder Elongase desaturiert und/oder elongiert oder nur desaturiert und anschließend über eine Elongase um mindestens zwei Kohlenstoffatome verlängert werden. Nach einer Elongationsrunde führt diese Enzymaktivität entweder ausgehend von C_{16}-Fettsäuren zu C_{18}-Fettsäuren oder ausgehend von C_{18}-Fettsäuren zu C_{20}-Fettsäuren, und nach zwei Elongationsrunden ausgehend von C_{16}-Fettsäuren zu C_{20}-Fettsäuren. Die Aktivität der im erfindungsgemäßen Verfahren verwendeten Desaturasen und Elongasen führt vorzugsweise zu C_{20}- und/oder C_{22}-Fettsäuren vorteilhaft mit mindestens zwei oder drei Doppelbindungen im Fettsäuremolekül, vorzugsweise mit vier, fünf oder sechs Doppelbindungen, besonders bevorzugt zu C_{22}-Fettsäuren mit mindestens fünf Doppelbindungen im Fettsäuremolekül. Besonders bevorzugt als Produkte des erfindungsgemäßen Verfahrens sind Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure. Die C_{18}-Fettsäuren mit mindestens zwei Doppelbindungen in der Fettsäure können durch die erfindungsgemäße enzymatische Aktivität in Form der freien Fettsäure oder in Form der Ester, wie Phospholipide, Glycolipide, Sphingolipide, Phosphoglyceride, Monoacylglycerin, Diacylglycerin oder Triacylglycerin, verlängert werden.

Der bevorzugte Biosyntheseort von Fettsäuren, Ölen, Lipiden oder Fette in den vorteilhaft verwendeten Pflanzen ist beispielsweise im allgemeinen der Samen oder Zellschichten des Samens, so dass eine samenspezifische Expression der im Verfahren verwen-

deten Nukleinsäuren sinnvoll ist. Es ist jedoch naheliegend, dass die Biosynthese von Fettsäuren, Ölen oder Lipiden nicht auf das Samengewebe beschränkt sein muss, sondern auch in allen übrigen Teilen der Pflanze - beispielsweise in Epidermiszellen oder in den Knollen - gewebespezifisch erfolgen kann. Vorteilhaft findet die Synthese gemäß des erfinderischen Verfahrens im vegetativen (somatischen) Gewebe statt.

Durch das erfindungsgemäße Verfahren können die hergestellten mehrfach ungesättigten Fettsäuren in den im Verfahren verwendeten Pflanzen prinzipiell auf zwei Arten erhöht werden. Es kann vorteilhaft der Pool an freien mehrfach ungesättigten Fettsäuren und/oder der Anteil der über das Verfahren hergestellten veresterten mehrfach ungesättigten Fettsäuren erhöht werden. Vorteilhaft wird durch das erfindungsgemäße Verfahren der Pool an veresterten mehrfach ungesättigten Fettsäuren in den transgenen Pflanzen erhöht, vorteilhaft in Form der Phosphatidylester und/oder Triacylester.

Die im erfindungsgemäßen Verfahren verwendeten Sequenzen werden einzeln in Expressionskonstrukte kloniert oder auf einem gemeinsamen rekombinanten Nukleinsäuremolekül bereitgestellt und zum Einbringen und zur Expression in Organismen verwendet. Diese Expressionskonstrukte ermöglichen eine optimale Synthese der im erfindungsgemäßen Verfahren produzierten mehrfach ungesättigten Fettsäuren.

Die im Verfahren verwendeten Nukleinsäuren können nach Einbringung in eine Pflanze oder Pflanzenzelle entweder auf einem separaten Plasmid liegen oder vorteilhaft in das Genom der Wirtszelle integriert sein. Bei Integration in das Genom kann die Integration zufallsgemäß sein oder durch derartige Rekombination erfolgen, dass das native Gen durch die eingebrachte Kopie ersetzt wird, wodurch die Produktion der gewünschten Verbindung durch die Zelle moduliert wird, oder durch Verwendung eines Gens in trans, so dass das Gen mit einer funktionellen Expressionseinheit, welche mindestens eine die Expression eines Gens gewährleistende Sequenz und mindestens eine die Polyadenylierung eines funktionell transkribierten Gens gewährleistende Sequenz enthält, funktionell verbunden ist. Vorteilhaft werden die Nukleinsäuresequenzen über Multiexpressionskassetten oder Konstrukte zur multiparallelen Expression in die Pflanzen gebracht, d.h. die Nukleinsäuresequenzen liegen in einer gemeinsamen Expressionseinheit vor.

Es kann im Nukleinsäurekonstrukt mehr als eine Nukleinsäuresequenz kodierend für ein Polypeptid mit der enzymatischen Aktivität einer Δ-12-Desaturase, Δ-4-Desaturase, Δ-5-Desaturase, Δ-6-Desaturase, Δ-5-Elongase, Δ-6-Elongase und/oder ω-3-Desaturase enthalten sein. Es können auch mehrere Kopien einer Nukleinsäuresequenz kodierend für ein Polypeptid mit der enzymatischen Aktivität einer Δ-12-Desaturase, Δ-4-Desaturase, Δ-5-Desaturase, Δ-6-Desaturase, Δ-5-Elongase, Δ-6-Elongase und/oder ω-3-Desaturase enthalten sein.

Zum Einbringen werden die im Verfahren verwendeten Nukleinsäuren vorteilhaft einer Amplifikation und Ligation in bekannter Weise unterworfen. Vorzugsweise geht man in Anlehnung an das Protokoll der Pfu-DNA-Polymerase oder eines Pfu/Taq-DNA-Polymerasegemisches vor. Die Primer werden in Anlehnung an die zu amplifizierende Sequenz gewählt. Zweckmäßigerweise sollten die Primer so gewählt werden, dass das Amplifikat die gesamte kodogene Sequenz vom Start- bis zum Stop-Kodon umfasst. Im Anschluss an die Amplifikation wird das Amplifikat zweckmäßigerweise analysiert. Beispielsweise kann die Analyse nach gelelektrophoretischer Auftrennung hinsichtlich Qualität und Quantität erfolgen. Im Anschluss kann das Amplifikat nach einem Standardprotokoll gereinigt werden (z.B. Qiagen). Ein Aliquot des gereinigten Amplifikats steht dann für die nachfolgende Klonierung zur Verfügung. Geeignete Klonierungsvektoren sind dem Fachmann allgemein bekannt. Hierzu gehören insbesondere Vektoren, die in mikrobiellen Systemen replizierbar sind, also vor allem Vektoren, die eine effiziente Klonierung in Hefen oder Pilze gewährleisten, und die die stabile Transformation von Pflanzen ermöglichen. Zu nennen sind insbesondere verschiedene für die T-DNA-vermittelte Transformation geeignete, binäre und co-integrierte Vektorsysteme. Derartige Vektorsysteme sind in der Regel dadurch gekennzeichnet, dass sie zumindest die für die Agrobakterium-vermittelte Transformation benötigten vir-Gene sowie die T-DNA begrenzende Sequenzen (T-DNA-Border) beinhalten. Vorzugsweise umfassen diese Vektorsysteme auch weitere cis-regulatorische Regionen wie Promotoren und Terminatoren und/oder Selektionsmarker, mit denen entsprechend transformierte Organismen identifiziert werden können. Während bei co-integrierten Vektorsystemen vir-Gene und T-DNA-Sequenzen auf demselben Vektor angeordnet sind, basieren binäre Systeme auf wenigstens zwei Vektoren, von denen einer vir-Gene, aber keine T-DNA und ein zweiter T-

DNA, jedoch kein vir-Gen trägt. Dadurch sind letztere Vektoren relativ klein, leicht zu manipulieren und sowohl in E. coli als auch in Agrobacterium zu replizieren. Zu diesen binären Vektoren gehören Vektoren der Serien pBIB-HYG, pPZP, pBecks, pGreen. Erfindungsgemäß bevorzugt verwendet werden Bin19, pBI101, pBinAR, pGPTV und pCAMBIA. Eine Übersicht über binäre Vektoren und ihre Verwendung gibt Hellens et al. (2000) Trends in Plant Science 5: 446–451. Für die Vektorpräparation können die Vektoren zunächst mit Restriktionsendonuklease(n) linearisiert und dann in geeigneter Weise enzymatisch modifiziert werden. Im Anschluss wird der Vektor gereinigt und ein Aliquot für die Klonierung eingesetzt. Bei der Klonierung wird das enzymatisch geschnittene und erforderlichenfalls gereinigte Amplifikat mit ähnlich präparierten Vektorfragmenten unter Einsatz von Ligase kloniert. Dabei kann ein bestimmtes Nukleinsäurekonstrukt bzw. Vektor- oder Plasmidkonstrukt einen oder auch mehrere kodogene Genabschnitte aufweisen. Vorzugsweise sind die kodogenen Genabschnitte in diesen Konstrukten mit regulatorischen Sequenzen funktional verknüpft. Zu den regulatorischen Sequenzen gehören insbesondere pflanzliche Sequenzen wie die oben beschriebenen Promotoren und Terminatoren. Die Konstrukte lassen sich vorteilhafterweise in Mikroorganismen, insbesondere Escherichia coli und Agrobacterium tumefaciens, unter selektiven Bedingungen stabil propagieren und ermöglichen so einen Transfer von heterologer DNA in Pflanzen.

Unter der vorteilhaften Verwendung von Klonierungsvektoren können die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen und Nukleinsäurekonstrukte in Mikroorganismen und danach in Pflanzen eingebracht werden und damit bei der Pflanzentransformation verwendet werden, wie denjenigen, die veröffentlicht sind in und dort zitiert sind: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Kapitel 6/7, S. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. (1991) 42: 205-225. Die im Verfahren verwendeten Nukleinsäuren, Nukleinsäurekonstrukte und/oder Vektoren lassen sich damit zur gentechnologischen Veränderung eines breiten

Spektrums an Pflanzen verwenden, so dass diese bessere und/oder effizientere Produzenten von LCPUFAs werden.

Durch das Einbringen eines Δ-6-Desaturase-, Δ-6-Elongase-, Δ-5-Desaturase- und Δ-5-Elongase-Genes in eine Pflanze allein oder in Kombination mit anderen Genen kann nicht nur der Biosynthesefluss zum Endprodukt erhöht, sondern auch die entsprechende Triacylglycerin- und/oder Phosphatidylester-Zusammensetzung erhöht oder de novo geschaffen werden. Ebenso kann die Anzahl oder Aktivität anderer Gene, die am Import von Nährstoffen, die zur Biosynthese einer oder mehrerer Fettsäuren, Ölen, polaren und/oder neutralen Lipiden nötig sind, erhöht sein, so dass die Konzentration dieser Vorläufer, Cofaktoren oder Zwischenverbindungen innerhalb der Zellen oder innerhalb des Speicherkompartiments erhöht ist, wodurch die Fähigkeit der Zellen zur Produktion von PUFAs, wie im Folgenden beschrieben, weiter gesteigert wird. Durch Optimierung der Aktivität oder Erhöhung der Anzahl eines oder mehrerer der Δ-6-Desaturase-, Δ-6-Elongase-, Δ-5-Desaturase- und/oder Δ-5-Elongase-Gene, die an der Biosynthese dieser Verbindungen beteiligt sind, oder durch Zerstören der Aktivität einer oder mehrerer Gene, die am Abbau dieser Verbindungen beteiligt sind, kann es möglich sein, die Ausbeute, Produktion und/oder Effizienz der Produktion von Fettsäure- und Lipidmolekülen aus Organismen und vorteilhaft aus Pflanzen zu steigern.

Die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuremoleküle kodieren für Proteine oder Teile von diesen, wobei die Proteine oder das einzelne Protein oder Teile davon eine Aminosäuresequenz enthält, die ausreichend homolog zu einer Aminosäuresequenz ist, die in den Sequenzen SEQ ID NO. 65, SEQ ID NO. 2, SEQ ID NO. 172 oder SEQ ID NO. 52 und ggf. SEQ ID NO. 194 oder SEQ ID NO. 78 dargestellt ist, so dass die Proteine oder Teile davon noch eine Δ-6-Desaturase-, Δ-6-Elongase-, Δ-5-Desaturase- und/oder Δ-5-Elongase-Aktivität sowie ggf. eine Δ-4-Desaturase- und/oder ω-3-Desaturase-Aktivität aufweisen. Vorzugsweise haben die Proteine oder Teile davon, die von dem Nukleinsäuremolekül/den Nukleinsäuremolekülen kodiert wird/werden, noch seine/ihre wesentliche enzymatische Aktivität und die Fähigkeit, am Stoffwechsel von zum Aufbau von Zellmembranen oder Lipidkörperchen in Organismen vorteilhaft in Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese

Membranen teilzunehmen. Vorteilhaft sind die von den Nukleinsäuremolekülen kodierten Proteine zu mindestens etwa 60 % und bevorzugt mindestens etwa 70 %, 80 % oder 90 % und besonders bevorzugt mindestens etwa 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % oder mehr identisch zu den in SEQ ID NO. 65, SEQ ID NO. 2, SEQ ID NO. 172, SEQ ID NO. 52, SEQ ID NO. 194 oder SEQ ID NO. 78 dargestellten Aminosäuresequenzen. Im Sinne der Erfindung ist unter Homologie oder homolog, Identität oder identisch zu verstehen.

Die Homologie wurde über den gesamten Aminosäure- bzw. Nukleinsäuresequenzbereich berechnet. Für den Vergleich verschiedener Sequenzen stehen dem Fachmann eine Reihe von Programmen zur Verfügung, die auf verschiedenen Algorithmen beruhen. Dabei liefern die Algorithmen von Needleman und Wunsch oder Smith und Waterman besonders zuverlässige Ergebnisse. Für die Sequenzvergleiche wurde das Programm PileUp verwendet (J. Mol. Evolution (1987) 25: 351-360; Higgins et al. (1989) CABIOS 5: 151–153) oder die Programme Gap und BestFit (Needleman and Wunsch (1970) J. Mol. Biol. 48: 443-453 und Smith and Waterman (1981) Adv. Appl. Math. 2: 482-489), die im GCG Software-Packet (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)) enthalten sind. Die oben in Prozent angegebenen Sequenzhomologiewerte wurden mit dem Programm GAP über den gesamten Sequenzbereich mit folgenden Einstellungen ermittelt: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 und Average Mismatch: 0.000. Diese Einstellungen wurden, falls nicht anders angegeben, immer als Standardeinstellungen für Sequenzvergleiche verwendet.

Unter wesentlicher enzymatischer Aktivität der im erfindungsgemäßen Verfahren verwendeten ω-3-Desaturase, Δ-6-Desaturase, Δ-6-Elongase, Δ-5-Elongase, Δ-4-Desaturase und/oder Δ-5-Desaturase ist zu verstehen, dass sie im Vergleich zu den durch die Sequenz mit der SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 kodierten Proteinen/Enzymen noch eine enzymatische Aktivität von mindestens 10 %, bevorzugt von mindestens 20 %, besonders bevorzugt von mindestens 30 % und am meisten bevorzugt von mindestens 40, 50 oder 60 % aufweisen und damit am Stoffwechsel von zum Aufbau von Fettsäuren, vor-

teilhaft Fettsäureestern wie Phosphatidylestern und/oder Triacylglyceridestern, in einer Pflanze oder Pflanzenzelle notwendigen Verbindungen oder am Transport von Molekülen über Membranen teilnehmen können.

Vorteilhaft im Verfahren verwendbare Nukleinsäuren stammen aus Bakterien, Pilzen, Diatomeen, Tieren wie Caenorhabditis oder Oncorhynchus oder Pflanzen wie Algen oder Moosen wie den Gattungen Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Pytium irregulare, Mantoniella, Ostreococcus, Isochrysis, Aleurita, Muscarioides, Mortierella, Borago, Phaeodactylum, Crypthecodinium, speziell aus den Gattungen und Arten Pytium irregulare, Oncorhynchus mykiss, Xenopus laevis, Ciona intestinalis, Thalassiosira pseudonona, Mantoniella squamata, Ostreococcus sp., Ostreococcus tauri, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricornutum, Caenorhabditis elegans oder besonders vorteilhaft aus Pytium irregulare, Thraustochytrium sp. und/oder Ostreococcus tauri.

Im erfindungsgemäßen Verfahren können zusätzlich Nukleotidsequenzen verwendet werden, die für eine Δ-12-Desaturase, Δ-9-Elongase oder Δ-8-Desaturase kodieren. Die im Verfahren verwendeten Nukleinsäuresequenzen werden vorteilhaft in einer Expressionskassette, die die Expression der Nukleinsäuren in Pflanzen ermöglicht, eingebracht.

Die Nukleinsäuresequenzen, die für die Δ-12-Desaturase, ω-3-Desaturase, Δ-9-Elongase, Δ-6-Desaturase, Δ-8-Desaturase, Δ-6-Elongase, Δ-5-Desaturase, Δ-5-Elongase oder Δ-4-Desaturase kodieren, werden mit einem oder mehreren Regulationssignalen zur Erhöhung der Genexpression funktionell verknüpft. Diese regulatorischen Sequenzen sollen die gezielte Expression der Gene ermöglichen. Dies kann beispielsweise je nach Pflanze bedeuten, dass das Gen erst nach Induktion exprimiert und/oder überexprimiert wird, oder dass es sofort exprimiert und/oder überexprimiert wird. Vorteilhaft werden Sequenzen für die Expression verwendet, die eine konstitutive Expression ermöglichen, wie der CaMV35S-, CaMV36S-, CaMV35Smas-, nos-, mas-, ubi-, stpt-, lea- oder Super-Promotor. Bevorzugt erfolgt die Expression im vegetativen Gewebe wie oben

beschrieben. In einer anderen bevorzugten Ausführungsform erfolgt die Expression im Samen.

Beispielsweise handelt es sich bei diesen regulatorischen Sequenzen um Sequenzen, an die Induktoren oder Repressoren binden und so die Expression der Nukleinsäure regulieren. Zusätzlich zu den Regulationssequenzen, die in ihrem natürlichen Locus nicht mit den Nukleinsäuresequenzen verknüpft sind, oder anstelle dieser Sequenzen kann die natürliche Regulation dieser Sequenzen vor den eigentlichen Strukturgenen noch vorhanden sein und gegebenenfalls genetisch verändert worden sein, so dass die natürliche Regulation ausgeschaltet und die Expression der Gene erhöht ist. Das Genkonstrukt kann außerdem vorteilhafterweise auch eine oder mehrere sogenannte "Enhancer-Sequenzen" funktionell verknüpft mit dem Promotor enthalten, die eine erhöhte Expression der Nukleinsäuresequenz ermöglichen. Auch am 3'-Ende der DNA-Sequenzen können zusätzliche vorteilhafte Sequenzen inseriert werden wie weitere regulatorische Elemente oder Terminatoren. Vorteilhafte Terminatoren sind beispielweise virale Terminatoren wie der 35S-Terminator oder andere. Die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen können in einer oder mehreren Kopien in der Expressionskassette (= Genkonstrukt) enthalten sein. Vorteilhaft liegt nur jeweils eine Kopie der Gene in der Expressionskassette vor. Dieses Genkonstrukt oder die Genkonstrukte können gleichzeitig oder nacheinander in die Pflanze eingebracht werden und zusammen im Wirtsorganismus exprimiert werden. Dabei kann das Genkonstrukt oder die Genkonstrukte in einem oder mehreren Vektoren inseriert sein und frei in der Zelle vorliegen oder aber im Genom inseriert sein. Es ist vorteilhaft für die Insertion weiterer Gene in die Pflanze, wenn die zu exprimierenden Gene zusammen in einem Genkonstrukt vorliegen. Es ist aber auch möglich, jeweils ein Genkonstrukt enthaltend eine Nukleinsäuresequenz in eine Pflanze einzuführen und die so erhaltenen Pflanzen miteinander zu verkreuzen, um Nachkommen zu erhalten, die alle Genkonstrukte gemeinsam enthalten.

Die regulatorischen Sequenzen bzw. Faktoren können dabei wie oben beschrieben vorzugsweise die Genexpression der eingeführten Gene positiv beeinflussen und dadurch erhöhen. So kann eine Verstärkung der regulatorischen Elemente vorteilhafterweise auf

der Transkriptionsebene erfolgen, indem starke Transkriptionssignale wie Promotoren und/oder "Enhancer" verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert wird.

Um eine stabile Integration der Biosynthesegene in die transgene Pflanze über mehrere Generation sicherzustellen, sollte jede der im Verfahren verwendeten Nukleinsäuren, die für die Δ-6-Desaturase, Δ-6-Elongase, Δ-5-Desaturase oder Δ-5-Elongase und ggf. für die ω-3-Desaturase oder Δ-4-Desaturase kodieren, unter der Kontrolle eines eigenen Promotors exprimiert werden. Dieser kann für jede der Sequenzen gleich oder unterschiedlich sein. Die Expressionskassette ist dabei vorteilhaft so aufgebaut, dass einem Promotor eine geeignete Schnittstelle zur Insertion der zu exprimierenden Nukleinsäure folgt, die vorteilhaft in einem Polylinker liegt. Hinter dem Polylinker kann ggf. ein Terminator liegen. Diese Abfolge wiederholt sich mehrfach, bevorzugt drei-, vier-, fünf- oder sechsmal, so dass bis zu sechs Gene in einem Konstrukt zusammengeführt werden und so zur Expression in die transgene Pflanze eingebracht werden können.
Die Nukleinsäuresequenzen werden zur Expression über die geeignete Schnittstelle beispielsweise im Polylinker hinter den Promotor inseriert. Vorteilhaft hat jede Nukleinsäuresequenz ihren eigenen Promotor und gegebenenfalls ihren eigenen Terminator. Es ist aber auch möglich, mehrere Nukleinsäuresequenzen hinter einem Promotor und ggf. vor einem Terminator zu inserieren. Dabei ist die Insertionsstelle bzw. die Abfolge der inserierten Nukleinsäuren in der Expressionskassette nicht von entscheidender Bedeutung, das heißt eine Nukleinsäuresequenz kann an erster oder letzter Stelle in der Kassette inseriert sein, ohne dass durch die die Position die Expression wesentlich beeinflusst wird. Es können in der Expressionskassette in einer vorteilhaften Ausführungsform unterschiedliche Promotoren wie beispielsweise der USP-, LegB4 oder DC3-Promotor und unterschiedliche Terminatoren verwendet werden. In einer weiteren vorteilhaften Ausführungsform können auch identische Promotoren wie der CaMV35S-Promotor verwendet werden.

Wie oben beschrieben sollte die Transkription der eingebrachten Gene vorteilhaft durch geeignete Terminatoren am 3'-Ende der eingebrachten Biosynthesegene (hinter dem Stopcodon) abgebrochen werden. Verwendet werden kann hier z.B. der OCS1- oder der

35SCaMV-Terminator. Wie auch für die Promotoren, sollten hier für jedes Gen unterschiedliche Terminatorsequenzen verwendet werden.

Das Genkonstrukt kann, wie oben beschrieben, auch weitere Gene umfassen, die in die Organismen eingebracht werden sollen. Es ist möglich und vorteilhaft, in die Wirtspflanzen Regulationsgene, wie Gene für Induktoren, Repressoren oder Enzyme, welche durch ihre Enzymaktivität in die Regulation eines oder mehrerer Gene eines Biosynthesewegs eingreifen, einzubringen und darin zu exprimieren. Diese Gene können heterologen oder homologen Ursprungs sein. Weiterhin können vorteilhaft im Nukleinsäurekonstrukt bzw. Genkonstrukt weitere Biosynthesegene des Fettsäure- oder Lipidstoffwechsels enthalten sein oder aber diese Gene können auf einem weiteren oder mehreren weiteren Nukleinsäurekonstrukten liegen. Vorteilhaft werden als Biosynthesegene des Fettsäure- oder Lipidstoffwechsels ein oder mehrere Gene ausgewählt aus der Gruppe Acyl-CoA-Dehydrogenase(n), Acyl-ACP(= acyl carrier protein)–Desaturase(n), Acyl–ACP–Thioesterase(n), Fettsäure–Acyl–Transferase(n), Acyl-CoA:Lysophospholipid-Acyltransferase(n), Fettsäure–Synthase(n), Fettsäure–Hydroxylase(n), Acetyl-Coenzym A-Carboxylase(n), Acyl–Coenzym A–Oxidase(n), Fettsäure–Desaturase(n), Fettsäure–Acetylenase(n), Lipoxygenase(n), Triacylglycerol–Lipase(n), Allenoxid–Synthase(n), Hydroperoxid–Lyase(n) oder Fettsäure–Elongase(n) oder Kombinationen davon verwendet. Besonders vorteilhafte Nukleinsäuresequenzen sind Biosynthesegene des Fettsäure– oder Lipidstoffwechsels ausgewählt aus der Gruppe der Acyl-CoA:Lysophospholipid-Acyltransferase, Δ-8-Desaturase, Δ-9-Desaturase, Δ-12-Desaturase und/oder Δ-9-Elongase.

Dabei können die vorgenannten Nukleinsäuren bzw. Gene in Kombination mit anderen Elongasen und Desaturasen in Expressionskassetten, wie den vorgenannten, kloniert werden und zur Transformation von Pflanzen mit Hilfe von Agrobakterium eingesetzt werden.

Der in dieser Beschreibung verwendete Begriff "Vektor" steht für ein Nukleinsäuremolekül, das eine andere Nukleinsäure transportieren kann, an welche es gebunden ist. Ein Vektortyp ist ein "Plasmid", eine zirkuläre doppelsträngige DNA-Schleife, in die zu-

sätzlichen DNA-Segmente ligiert werden können. Ein weiterer Vektortyp ist ein viraler Vektor, wobei zusätzliche DNA-Segmente in das virale Genom ligiert werden können. Bestimmte Vektoren können in einer Wirtszelle, in die sie eingebracht worden sind, autonom replizieren (z.B. Bakterienvektoren mit bakteriellem Replikationsursprung). Andere Vektoren werden vorteilhaft beim Einbringen in die Wirtszelle in das Genom einer Wirtszelle integriert und dadurch zusammen mit dem Wirtsgenom repliziert. Zudem können bestimmte Vektoren die Expression von Genen, mit denen sie funktionsfähig verbunden sind, steuern. Diese Vektoren werden hier als "Expressionsvektoren" bezeichnet. Gewöhnlich haben Expressionsvektoren, die für DNA-Rekombinationstechniken geeignet sind, die Form von Plasmiden. In der vorliegenden Beschreibung können "Plasmid" und "Vektor" austauschbar verwendet werden, da das Plasmid die am häufigsten verwendete Vektorform ist. Die Erfindung soll jedoch auch andere Expressionsvektorformen, wie virale Vektoren, die ähnliche Funktionen ausüben, umfassen. Ferner soll der Begriff Vektor auch andere Vektoren, die dem Fachmann bekannt sind, wie Phagen, Viren wie SV40, CMV, TMV, Transposons, IS-Elemente, Phasmide, Phagemide, Cosmide, lineare oder zirkuläre DNA, umfassen.

Die im Verfahren vorteilhaft verwendeten rekombinanten Expressionsvektoren umfassen die im Verfahren verwendeten Nukleinsäuresequenzen oder das oben beschriebene Genkonstrukt in einer Form, die sich zur Expression der verwendeten Nukleinsäuren in einer Wirtszelle eignet, was bedeutet, dass die rekombinanten Expressionsvektoren eine oder mehrere Regulationssequenzen, die auf der Basis der zur Expression zu verwendenden Wirtszellen ausgewählt ist und die mit der zu exprimierenden Nukleinsäuresequenz funktionsfähig verbunden ist, umfasst. In einem rekombinanten Expressionsvektor bedeutet "funktionsfähig verbunden", dass die Nukleotidsequenz von Interesse derart an die Regulationssequenz(en) gebunden ist, dass die Expression der Nukleotidsequenz ermöglicht wird und sie aneinander gebunden sind, so dass beide Sequenzen die vorhergesagte, der Sequenz zugeschriebene Funktion erfüllen (z.B. in einem In-vitro-Transkriptions-/Translationssystem oder in einer Wirtszelle, wenn der Vektor in die Wirtszelle eingebracht wird). Der Begriff "Regulationssequenz" soll Promotoren, Enhancer und andere Expressionskontrollelemente (z.B. Polyadenylierungssignale) umfassen. Diese Regulationssequenzen sind z.B. beschrieben in Goeddel: Gene Expression Tech-

nology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), oder siehe: Gruber und Crosby, in: Methods in Plant Molecular Biology and Biotechnolgy, CRC Press, Boca Raton, Florida, Hrsgb.: Glick und Thompson, Kapitel 7, 89-108, einschließlich der Literaturstellen darin. Regulationssequenzen umfassen solche, welche die konstitutive Expression einer Nukleotidsequenz in vielen Wirtszelltypen steuern, und solche, welche die direkte Expression der Nukleotidsequenz nur in bestimmten Wirtszellen unter bestimmten Bedingungen steuern. Der Fachmann weiß, dass die Gestaltung des Expressionsvektors von Faktoren, wie der Auswahl der zu transformierenden Wirtszelle, dem gewünschten Ausmaß der Expression des Proteins, usw. abhängen kann.

Die verwendeten rekombinanten Expressionsvektoren können zur Expression der im Verfahren verwendeten Nukleinsäuresequenzen so gestaltet sein, dass sie in prokaryotische Zwischenwirte transformiert werden können und schließlich nach Einbringung in die Pflanzen die Expression der Gene in diesen ermöglichen. Dies ist vorteilhaft, da häufig Zwischenschritte der Vektorkonstruktion der Einfachheit halber in Mikroorganismen durchgeführt werden. Beispielsweise können die Δ-6-Desaturase-, Δ-6-Elongase-, Δ-5-Desaturase- und/oder Δ-5-Elongase-Gene in bakteriellen Zellen, Insektenzellen (unter Verwendung von Baculovirus-Expressionsvektoren), Hefe- und anderen Pilzzellen (siehe Romanos, M.A., et al. (1992) Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Hrsgb., S. 396-428: Academic Press: San Diego; und van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Hrsgb., S. 1-28, Cambridge University Press: Cambridge), Algen (Falciatore et al. (1999) Marine Biotechnology.1: (3):239-251), Ciliaten, mit Vektoren nach einem Transformationsverfahren, wie beschrieben in WO 98/01572, sowie bevorzugt in Zellen vielzelliger Pflanzen (siehe Schmidt, R. und Willmitzer, L. (1988) "High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants" Plant Cell Rep.:583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, Kapitel 6/7, S.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press (1993), 128-43; Potrykus (1991) Annu. Rev. Plant

Physiol. Plant Molec. Biol. 42: 205-225 (und darin zitierte Literaturstellen)) exprimiert werden. Geeignete Wirtszellen werden ferner erörtert in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Der rekombinante Expressionsvektor kann alternativ, zum Beispiel unter Verwendung von T7-Promotor-Regulationssequenzen und T7-Polymerase, in vitro transkribiert und translatiert werden.

Die Expression von Proteinen in Prokaryonten erfolgt meist mit Vektoren, die konstitutive oder induzierbare Promotoren enthalten, welche die Expression von Fusions- oder nicht-Fusionsproteinen steuern. Typische Fusions-Expressionsvektoren sind u.a. pGEX (Pharmacia Biotech Inc; Smith, D.B., und Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) und pRIT5 (Pharmacia, Piscataway, NJ), bei denen Glutathion-S-Transferase (GST), Maltose E-bindendes Protein bzw. Protein A an das rekombinante Zielprotein fusioniert wird.

Beispiele für geeignete induzierbare nicht-Fusions-E. coli-Expressionsvektoren sind u.a. pTrc (Amann et al. (1988) Gene 69:301-315) und pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Kalifornien (1990) 60-89). Die Zielgenexpression vom pTrc-Vektor beruht auf der Transkription durch Wirts-RNA-Polymerase von einem Hybrid-trp-lac-Fusionspromotor. Die Zielgenexpression aus dem pET 11d-Vektor beruht auf der Transkription von einem T7-gn10-lac-Fusions-Promotor, die von einer koexprimierten viralen RNA-Polymerase (T7 gn1) vermittelt wird. Diese virale Polymerase wird von den Wirtsstämmen BL21 (DE3) oder HMS174 (DE3) von einem residenten λ-Prophagen bereitgestellt, der ein T7 gn1-Gen unter der Transkriptionskontrolle des lacUV 5-Promotors birgt.

Andere in prokaryotischen Organismen geeignete Vektoren sind dem Fachmann bekannt, diese Vektoren sind beispielsweise in E. coli pLG338, pACYC184, die pBR–Reihe, wie pBR322, die pUC–Reihe, wie pUC18 oder pUC19, die M113mp–Reihe, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCI, in Streptomyces pIJ101, pIJ364, pIJ702 oder pIJ361, in Bacillus pUB110, pC194 oder pBD214, in Corynebacterium pSA77 oder pAJ667.

Bei einer weiteren Ausführungsform ist der Expressionsvektor ein Hefe-Expressionsvektor. Beispiele für Vektoren zur Expression in der Hefe S. cerevisiae umfassen pYe-Desaturasec1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan und Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) sowie pYES2 (Invitrogen Corporation, San Diego, CA). Vektoren und Verfahren zur Konstruktion von Vektoren, die sich zur Verwendung in anderen Pilzen, wie den filamentösen Pilzen, eignen, umfassen diejenigen, die eingehend beschrieben sind in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., Hrsgb., S. 1-28, Cambridge University Press: Cambridge, oder in: More Gene Manipulations in Fungi (J.W. Bennet & L.L. Lasure, Hrsgb., S. 396-428: Academic Press: San Diego). Weitere geeignete Hefevektoren sind beispielsweise pAG-1, YEp6, YEp13 oder pEMBLYe23.

Alternativ können die im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen in Insektenzellen unter Verwendung von Baculovirus-Expressionsvektoren exprimiert werden. Baculovirus-Vektoren, die zur Expression von Proteinen in gezüchteten Insektenzellen (z.B. Sf9-Zellen) verfügbar sind, umfassen die pAc-Reihe (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) und die pVL-Reihe (Lucklow und Summers (1989) Virology 170:31-39).

Die oben genannten Vektoren bieten nur einen kleinen Überblick über mögliche geeignete Vektoren. Weitere Plasmide sind dem Fachmann bekannt und sind zum Beispiel beschrieben in: Cloning Vectors (Hrsgb. Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Weitere geeignete Expressionssysteme für prokaryotische und eukaryotische Zellen siehe in den Kapiteln 16 und 17 von Sambrook, J., Fritsch, E.F., und Maniatis, T., Molecular Cloning: A Laboratory Manual, 2. Auflage, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Auch können die im Verfahren verwendeten Gen in einzelligen Pflanzenzellen (wie Algen), siehe Falciatore et al. (1999) Marine Biotechnology 1 (3):239-251 und darin zitierte

Literaturangaben, und Pflanzenzellen aus höheren Pflanzen (z.B. Spermatophyten, wie Feldfrüchten) exprimiert werden. Beispiele für Pflanzen-Expressionsvektoren umfassen solche, die eingehend beschrieben sind in: Becker, D., Kemper, E., Schell, J., und Masterson, R. (1992) Plant Mol. Biol. 20:1195-1197; und Bevan, M.W. (1984) Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press, 1993, S. 15-38.

Eine Pflanzen-Expressionskassette enthält vorzugsweise Regulationssequenzen, welche die Genexpression in Pflanzenzellen steuern können und funktionsfähig verbunden sind, so dass jede Sequenz ihre Funktion, wie Termination der Transkription, erfüllen kann, beispielsweise Polyadenylierungssignale. Bevorzugte Polyadenylierungssignale sind diejenigen, die aus Agrobacterium tumefaciens-T-DNA stammen, wie das als Octopinsynthase bekannte Gen 3 des Ti-Plasmids pTiACH5 (Gielen et al. (1984) EMBO J. 3. 835ff.) oder funktionelle Äquivalente davon, aber auch alle anderen in Pflanzen funktionell aktiven Terminatoren sind geeignet.

Da die Regulation der Pflanzengenexpression sehr oft nicht auf die Transkriptionsebene beschränkt ist, enthält eine Pflanzen-Expressionskassette vorzugsweise andere funktionsfähig verbundene Sequenzen, wie Translationsenhancer, beispielsweise die Overdrive-Sequenz, welche die 5'-untranslatierte Leader-Sequenz aus Tabakmosaikvirus, die das Protein/RNA-Verhältnis erhöht, enthält (Gallie et al. (1987) Nucl. Acids Research 15:8693-8711).

Die Pflanzengenexpression muss wie oben beschrieben funktionsfähig mit einem geeigneten Promotor verbunden sein, der die Genexpression steuert. Vorteilhaft nutzbare Promotoren sind konstitutive Promotoren (Benfey et al., EMBO J. (1989) 8: 2195-2202), wie diejenigen, die von Pflanzenviren stammen, wie 35S CAMV (Franck et al. (1980) Cell 21: 285-294), 19S CaMV (siehe auch US 5352605 und WO 84/02913) oder Pflanzenpromotoren, wie der in US 4,962,028 beschriebene der kleinen Untereinheit der Rubisco.

Andere bevorzugte Sequenzen für die Verwendung zur funktionsfähigen Verbindung in Pflanzengenexpressions-Kassetten sind Targeting-Sequenzen, die zur Steuerung des Genproduktes in sein entsprechendes Zellkompartiment), beispielsweise in die Vakuole, den Zellkern, alle Arten von Plastiden, wie Amyloplasten, Chloroplasten, Chromoplasten, den extrazellulären Raum, die Mitochondrien, das Endoplasmatische Retikulum, Ölkörper, Peroxisomen und andere Kompartimente von Pflanzenzellen; notwendig sind (siehe eine Übersicht in Kermode (1996) Crit. Rev. Plant Sci. 15 (4): 285-423 und darin zitierte Literaturstellen).

Vektor-DNA lässt sich in prokaryotische oder eukaryotische Zellen über herkömmliche Transformations- oder Transfektionstechniken einbringen. Die Begriffe "Transformation" und "Transfektion", Konjugation und Transduktion, wie hier verwendet, sollen eine Vielzahl von im Stand der Technik bekannten Verfahren zum Einbringen fremder Nukleinsäuren (z.B. DNA) in eine Wirtszelle, einschließlich Calciumphosphat- oder Calciumchlorid-Kopräzipitation, DEAE-Dextran-vermittelte Transfektion, Lipofektion, natürliche Kompetenz, chemisch vermittelter Transfer, Elektroporation oder Teilchenbeschuss, umfassen. Geeignete Verfahren zur Transformation oder Transfektion von Wirtszellen, einschließlich Pflanzenzellen, lassen sich finden in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2. Aufl., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) und anderen Labor-Handbüchern, wie Methods in Molecular Biology, 1995, Bd. 44, Agrobacterium protocols, Hrsgb: Gartland und Davey, Humana Press, Totowa, New Jersey.

Der Begriff "Nukleinsäure(molekül)", wie hier verwendet, umfasst in einer vorteilhaften Ausführungsform zudem die am 3'- und am 5'-Ende des kodierenden Genbereichs gelegene untranslatierte Sequenz: mindestens 500, bevorzugt 200, besonders bevorzugt 100 Nukleotide der Sequenz stromaufwärts des 5'-Endes des kodierenden Bereichs und mindestens 100, bevorzugt 50, besonders bevorzugt 20 Nukleotide der Sequenz stromabwärts des 3'-Endes des kodierenden Genbereichs. Ein "isoliertes" Nukleinsäuremolekül wird von anderen Nukleinsäuremolekülen abgetrennt, die in der natürlichen Quelle der Nukleinsäure vorliegen. Eine "isolierte" Nukleinsäure hat vorzugsweise keine Sequenzen, welche die Nukleinsäure in der genomischen DNA des Organismus, aus dem

die Nukleinsäure stammt, natürlicherweise flankieren (z.B. Sequenzen, die sich an den 5'- und 3'-Enden der Nukleinsäure befinden). Bei verschiedenen Ausführungsformen kann das im Verfahren vewendete isolierte Δ-6-Desaturase-, Δ-6-Elongase- oder Δ-5-Desaturase- sowie ggf. das ω-3-Desaturase- oder Δ-4-Desaturase-Molekül zum Beispiel weniger als etwa 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0,5 kb oder 0,1 kb an Nukleotidsequenzen enthalten, die natürlicherweise das Nukleinsäuremolekül in der genomischen DNA der Zelle, aus der die Nukleinsäure stammt, flankieren.

Die im Verfahren verwendeten Nukleinsäuremoleküle können unter Verwendung molekularbiologischer Standardtechniken und der hier bereitgestellten Sequenzinformation isoliert werden. Auch kann mit Hilfe von Vergleichsalgorithmen beispielsweise eine homologe Sequenz oder homologe, konservierte Sequenzbereiche auf DNA- oder Aminosäureebene identifiziert werden. Diese können als Hybridisierungssonde in Standard-Hybridisierungstechniken (wie z.B. beschrieben in Sambrook et al., Molecular Cloning: A Laboratory Manual. 2. Aufl., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) zur Isolierung weiterer im Verfahren nützlicher Nukleinsäuresequenzen verwendet werden. Überdies lassen sich die im Verfahren verwendeten Nukleinsäuremoleküle oder Teile von diesen durch Polymerasekettenreaktion isolieren, wobei Oligonukleotidprimer auf der Basis dieser Sequenz oder von Teilen davon verwendet werden (z.B. kann ein Nukleinsäuremolekül umfassend die vollständige Sequenz oder einen Teil davon durch Polymerasekettenreaktion unter Verwendung von Oligonukleotidprimern isoliert werden, die auf der Basis dieser gleichen Sequenz erstellt worden sind). Zum Beispiel lässt sich mRNA aus Zellen isolieren (z.B. durch das Guanidiniumthiocyanat-Extraktionsverfahren von Chirgwin et al. (1979) Biochemistry 18:5294-5299) und cDNA mittels Reverser Transkriptase (z.B. Moloney-MLV-Reverse-Transkriptase, erhältlich von Gibco/BRL, Bethesda, MD, oder AMV-Reverse-Transkriptase, erhältlich von Seikagaku America, Inc., St.Petersburg, FL) herstellen. Synthetische Oligonukleotidprimer zur Amplifizierung mittels Polymerasekettenreaktion lassen sich auf der Basis einer der in SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 gezeigten Sequenzen oder mit Hilfe der in SEQ ID NO. 65, SEQ ID NO. 2, SEQ ID NO. 172, SEQ ID NO. 52, SEQ ID NO. 194 oder SEQ ID NO. 78 dargestellten Aminosäuresequenzen erstellen. Eine erfin-

dungsgemäße Nukleinsäure kann unter Verwendung von cDNA oder alternativ von genomischer DNA als Matrize und geeigneten Oligonukleotidprimern nach Standard-PCR-Amplifikationstechniken amplifiziert werden. Die so amplifizierte Nukleinsäure kann in einen geeigneten Vektor kloniert werden und mittels DNA-Sequenzanalyse charakterisiert werden. Oligonukleotide können durch Standard-Syntheseverfahren, beispielsweise mit einem automatischen DNA-Synthesegerät, hergestellt werden.

Homologe der verwendeten Δ-5-Elongase-, ω-3-Desaturase-, Δ-6-Desaturase-, Δ-6-Elongase-, Δ-4-Desaturase- oder Δ-5-Desaturase-Nukleinsäuresequenzen mit der Sequenz SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 bedeutet beispielsweise allelische Varianten mit mindestens etwa 40, 50 oder 60 %, vorzugsweise mindestens etwa 60 oder 70 %, stärker bevorzugt mindestens etwa 70 oder 80 %, 90 % oder 95 % und noch stärker bevorzugt mindestens etwa 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % oder mehr Identität bzw. Homologie zu einer der in SEQ ID NO. 64, 66, 68 oder 70, zu einer der in SEQ ID NO. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 oder 41, zu einer der in SEQ ID NO. 171, 173, 175, 177, 179, 181 oder 183, zu einer der in SEQ ID NO. 51, 53 oder 55, zu einer der in SEQ ID NO. 193 oder 195 oder zu einer der in oder SEQ ID NO. 77, 79, 81, 83, 85, 87, 89, 91 oder 93, insbesondere der in SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 gezeigten Nukleotidsequenzen oder ihren Homologen, Derivaten oder Analoga oder Teilen davon. Weiterhin sind isolierte Nukleinsäuremoleküle einer Nukleotidsequenz umfasst, die an eine der in SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 gezeigten Nukleotidsequenzen oder einen Teil davon, z.B. unter stringenten Bedingungen hybridisieren. Unter einem Teil gemäß der Erfindung ist dabei zu verstehen, dass mindestens 25 Basenpaare (= bp), 50 bp, 75 bp, 100 bp, 125 bp oder 150 bp, bevorzugt mindestens 175 bp, 200 bp, 225 bp, 250 bp, 275 bp oder 300 bp, besonders bevorzugt 350 bp, 400 bp, 450 bp, 500 bp oder mehr Basenpaare für die Hybridisierung verwendet werden. Es kann auch vorteilhaft die Gesamtsequenz verwendet werden. Allelische Varianten umfassen insbesondere funktionelle Varianten, die sich durch Deletion, Insertion oder Substitution von Nukleotiden aus der in SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID

NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 dargestellten Sequenz erhalten lassen, wobei aber die Enzymaktivität der davon kodierten Proteine für die Insertion im wesentlichen beibehalten wird.

Für das erfindungsgemäße Verfahren vorteilhafte Nukleinsäuremoleküle können auf der Grundlage ihrer Homologie zu den hier offenbarten ω-3-Desaturase-, Δ-6-Desaturase-, Δ-5-Desaturase-, Δ-5-Elongase-, Δ-4-Desaturase- und/oder Δ-6-Elongase-Nukleinsäuresequenzen unter Verwendung der Sequenzen oder eines Teils davon als Hybridisierungssonde gemäß Standard-Hybridisierungstechniken unter stringenten Hybridisierungsbedingungen isoliert werden. Dabei können beispielsweise isolierte Nukleinsäuremoleküle verwendet werden, die mindestens 15 Nukleotide lang sind und unter stringenten Bedingungen mit den Nukleinsäuremolekülen, die eine Nukleotidsequenz der SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder Seq ID No. 77 umfassen, hybridisieren. Es können auch Nukleinsäuremoleküle mit mindestens 25, 50, 100, 250 oder mehr Nukleotiden verwendet werden.

Der Begriff "hybridisiert unter stringenten Bedingungen", wie hier verwendet, soll Hybridisierungs- und Waschbedingungen beschreiben, unter denen Nukleotidsequenzen, die mindestens 60 % homolog zueinander sind, gewöhnlich aneinander hybridisiert bleiben. Die Bedingungen sind vorzugsweise derart, dass Sequenzen, die mindestens etwa 65 %, bevorzugt mindestens etwa 70 % und besonders bevorzugt mindestens etwa 75 % oder stärker zueinander homolog sind, gewöhnlich aneinander hybridisiert bleiben. Diese stringenten Bedingungen sind dem Fachmann bekannt und lassen sich in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. finden. Ein bevorzugtes, nicht einschränkendes Beispiel für stringente Hybridisierungsbedingungen sind Hybridisierungen in 6 x Natriumchlorid/Natriumcitrat (sodium chloride/sodium citrate = SSC) bei etwa 45°C, gefolgt von einem oder mehreren Waschschritten in 0,2 x SSC, 0,1 % SDS bei 50 bis 65°C. Dem Fachmann ist bekannt, dass diese Hybridisierungsbedingungen sich je nach dem Typ der Nukleinsäure und, wenn beispielsweise organische Lösungsmittel vorliegen, hinsichtlich der Temperatur und der Konzentration des Puffers unterscheiden. Die Temperatur liegt beispielsweise unter "Standard-Hybridisierungsbedingungen" je nach dem Typ der Nukleinsäure zwischen

42°C und 58°C in wässrigem Puffer mit einer Konzentration von 0,1 bis 5 x SSC (pH 7,2). Falls organisches Lösungsmittel, zum Beispiel 50 % Formamid, im oben genannten Puffer vorliegt, ist die Temperatur unter Standardbedingungen etwa 42°C. Vorzugsweise sind die Hybridisierungsbedingungen für DNA:DNA-Hybride zum Beispiel 0,1 x SSC und 20°C bis 45°C, vorzugsweise 30°C bis 45°C. Vorzugsweise sind die Hybridisierungsbedingungen für DNA:RNA-Hybride zum Beispiel 0,1 x SSC und 30°C bis 55°C, vorzugsweise 45°C bis 55°C. Die vorstehend genannten Hybridisierungstemperaturen sind beispielsweise für eine Nukleinsäure mit etwa 100 bp (= Basenpaare) Länge und einem G + C-Gehalt von 50 % in Abwesenheit von Formamid bestimmt. Der Fachmann weiß, wie die erforderlichen Hybridisierungsbedingungen anhand von Lehrbüchern, wie dem vorstehend erwähnten oder aus den folgenden Lehrbüchern Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames und Higgins (Hrsgb.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Hrsgb.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford, bestimmt werden können.

Zur Bestimmung der prozentualen Homologie (= Identität) von zwei Aminosäuresequenzen (z.B. einer der Sequenzen der SEQ ID NO. 65, SEQ ID NO. 2, SEQ ID NO. 172, SEQ ID NO. 52, SEQ ID NO. 194 oder SEQ ID NO. 78) oder von zwei Nukleinsäuren (z.B. SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77) werden die Sequenzen untereinander geschrieben, um sie optimal vergleichen zu können (z.B. können Lücken in die Sequenz eines Proteins oder einer Nukleinsäure eingefügt werden, um ein optimales Alignment mit dem anderen Protein oder der anderen Nukleinsäure zu erzeugen). Die Aminosäurereste oder Nukleotide an den entsprechenden Aminosäurepositionen oder Nukleotidpositionen werden dann verglichen. Wenn eine Position in einer Sequenz durch den gleichen Aminosäurerest oder das gleiche Nukleotid wie die entsprechende Stelle in der anderen Sequenz belegt wird, dann sind die Moleküle an dieser Position homolog (d.h. Aminosäure- oder Nukleinsäure-"Homologie", wie hier verwendet, entspricht Aminosäure- oder Nukleinsäure-"Identität"). Die prozentuale Homologie zwischen den beiden Sequenzen ist eine Funktion der Anzahl an identischen Positionen, die den Sequenzen gemeinsam sind (d.h. % Homologie = Anzahl der identischen Positionen/Gesamtanzahl der Positionen

x 100). Die zur Bestimmung der Homologie verwendeten Programme bzw. Algorithmen sind oben beschrieben.

Ein isoliertes Nukleinsäuremolekül, das für eine im Verfahren verwendete ω-3-Desaturase, Δ-6-Desaturase, Δ-5-Desaturase, Δ-5-Elongase, Δ-4-Desaturase und/oder Δ-6-Elongase kodiert, die zu einer Proteinsequenz der SEQ ID NO. 65, SEQ ID NO. 2, SEQ ID NO. 172, SEQ ID NO. 52, SEQ ID NO. 194 oder SEQ ID NO. 78 homolog ist, kann durch Einbringen einer oder mehrerer Nukleotidsubstitutionen, -additionen oder -deletionen in eine Nukleotidsequenz der SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 erzeugt werden, so dass eine oder mehrere Aminosäuresubstitutionen, -additionen oder -deletionen in das kodierte Protein eingebracht werden. Mutationen können in eine der Sequenzen der SEQ ID NO. 64, SEQ ID NO. 1, SEQ ID NO. 171, SEQ ID NO. 51, SEQ ID NO. 193 oder SEQ ID NO. 77 durch Standardtechniken, wie stellenspezifische Mutagenese und PCR-vermittelte Mutagenese, eingebracht werden. Vorzugsweise werden konservative Aminosäuresubstitutionen an einer oder mehreren der vorhergesagten nicht-essentiellen Aminosäureresten hergestellt. Bei einer "konservativen Aminosäuresubstitution" wird der Aminosäurerest gegen einen Aminosäurerest mit einer ähnlichen Seitenkette ausgetauscht. Im Fachgebiet sind Familien von Aminosäureresten mit ähnlichen Seitenketten definiert worden. Diese Familien umfassen Aminosäuren mit basischen Seitenketten (z.B. Lysin, Arginin, Histidin), sauren Seitenketten (z.B. Asparaginsäure, Glutaminsäure), ungeladenen polaren Seitenketten (z.B. Glycin, Asparagin, Glutamin, Serin, Threonin, Tyrosin, Cystein), unpolaren Seitenketten, (z.B. Alanin, Valin, Leucin, Isoleucin, Prolin, Phenylalanin, Methionin, Tryptophan), beta-verzweigten Seitenketten (z.B. Threonin, Valin, Isoleucin) und aromatischen Seitenketten (z.B. Tyrosin, Phenylalanin, Tryptophan, Histidin). Ein vorhergesagter nicht-essentieller Aminosäurerest in einer ω-3-Desaturase, Δ-6-Desaturase, Δ-5-Desaturase, Δ-5-Elongase, Δ-4-Desaturase oder Δ-6-Elongase wird somit vorzugsweise durch einen anderen Aminosäurerest aus der gleichen Seitenkettenfamilie ausgetauscht. Alternativ können bei einer anderen Ausführungsform die Mutationen zufallsgemäß über die gesamte oder einen Teil der ω-3-Desaturase, Δ-6-Desaturase, Δ-5-Desaturase, Δ-5-Elongase, Δ-4-Desaturase oder Δ-6-Elongase kodierenden Sequenz eingebracht werden, z.B. durch Sättigungsmutagenese, und die resul-

tierenden Mutanten können nach der hier beschriebenen ω-3-Desaturase-, Δ-6-Desaturase-, Δ-5-Desaturase-, Δ-5-Elongase-, Δ-4-Desaturase- oder Δ-6-Elongase-Aktivität durchmustert werden, um Mutanten zu identifizieren, die die ω-3-Desaturase-, Δ-6-Desaturase-, Δ-5-Desaturase-, Δ-5-Elongase-, Δ-4-Desaturase- oder Δ-6-Elongase-Aktivität beibehalten haben. Nach der Mutagenese kann das kodierte Protein rekombinant exprimiert werden, und die Aktivität des Proteins kann z.B. unter Verwendung der hier beschriebenen Tests bestimmt werden.

Diese Erfindung wird durch die nachstehenden Beispiele weiter veranschaulicht, die nicht als beschränkend aufgefasst werden sollten. Der Inhalt sämtlicher in dieser Patentanmeldung zitierten Literaturstellen, Patentanmeldungen, Patente und veröffentlichten Patentanmeldungen ist hier durch Bezugnahme aufgenommen.

Die folgende Tabelle zeigt die Sequenzkennzahlen, wie sie in der Prioritätsanmeldung vom 21.02.2006 mit dem deutschen Anmeldeaktenzeichen 102006008030.0 verwendet wurden sowie den entsprechenden Sequenzkennzahlen in der vorliegenden Nachanmeldung. Die durch die SEQ ID No: 1 der Prioritätsanmeldung gekennzeichnete Nukleinsäuresequenz entspricht beispielsweise der durch die SEQ ID NO: 64 der Nachanmeldung gekennzeichneten Nukleinsäuresequenz.

Konkordanztabelle Sequenzkennzahlen der Prioritätsanmeldung und der Sequenzkennzahlen in der Nachanmeldung:

SEQ ID NO: Prioritätsanmeldung Deutsches Anmeldeaktenzeichen 102006008030.0	SEQ ID NO: vorliegende Nachanmeldung	Organism
1	64	Ostreococcus tauri
2	65	Ostreococcus tauri
3	1	Phytium irregulare
4	2	Phytium irregulare
5	171	Traustochytrium sp.
6	172	Traustochytrium sp.
7	51	Thraustochytrium ssp.

8	52	Thraustochytrium ssp.
9	193	Phytophthora infestans
10	194	Phytophthora infestans
11	77	Traustochytrium sp.
12	78	Traustochytrium sp.
13	109	Ostreococcus tauri
n.a.	110	Ostreococcus tauri
14	122	Ostreococcus tauri
n.a.	123	Ostreococcus tauri
15	143	Ostreococcus tauri
16	144	Ostreococcus tauri
17	161	Cauliflower mosaic virus
18	162	Cauliflower mosaic virus
19	163	Thalassiosira pseudonana
20	164	Thalassiosira pseudonana

Beispiele

Beispiel 1: Allgemeine Klonierungsverfahren

Die Klonierungsverfahren wie z.B. Restriktionsspaltungen, Agarose-Gelelektrophorese, Reinigung von DNA-Fragmenten, Transfer von Nukleinsäuren auf Nitrozellulose- und Nylon-Membranen, Verknüpfen von DNA-Fragmenten, Transformation von Escherichia coli-Zellen, Anzucht von Bakterien und die Sequenzanalyse rekombinanter DNA wurden wie bei Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) beschrieben durchgeführt.

Beispiel 2: Sequenzanalyse rekombinanter DNA

Die Sequenzierung rekombinanter DNA-Moleküle erfolgte mit einem Laserfluoreszenz-DNA-Sequenzierer der Firma ABI nach der Methode von Sanger (Sanger et al. (1977)

Proc. Natl. Acad. Sci. USA 74: 5463-5467). Fragmente resultierend aus einer Polymerase-Kettenreaktion wurden zur Vermeidung von Polymerasefehlern in zu exprimierenden Konstrukten sequenziert und überprüft.

Beispiel 3: Klonierung von Genen aus Ostreococcus tauri

In einer Ostreococcus tauri Sequenzdatenbank (genomische Sequenzen) konnte durch Suche nach konservierten Bereichen jeweils eine Sequenz kodierend für ein Protein mit Δ-5-Elongaseaktivität oder Δ-6-Elongase-Aktivität identifiziert werden. Es handelt sich dabei um die folgenden Sequenzen:

Gen-Name	SEQ ID	Aminosäuren
OtELO1.1, (d-6-Elongase)	SEQ ID NO. 143	292
OtELO2.1, (Δ-5-Elongase)	SEQ ID NO. 109	300

OtElo2.1 weist die höchste Ähnlichkeit zu einer Elongase aus Danio rerio auf (GenBank AAN77156; ca. 26 % Identität), während OtElo1.1 die größte Ähnlichkeit zur Elongase aus Physcomitrella (PSE) (ca. 36 % Identität) aufweist (Alignments wurden mit dem tBLASTn-Aalgorithmus (Altschul et al. (1990) J. Mol. Biol. 215: 403 – 410) durchgeführt).

Die Klonierung der Elongasen wurde wie folgt durchgeführt:

40 ml einer Ostreococcus tauri Kultur in der stationären Phase wurden abzentrifugiert und in 100 µl Aqua bidest resuspendiert und bei –20°C gelagert. Mit Hilfe des PCR-Verfahrens wurden die entsprechenden genomischen DNAs amplifiziert. Die entsprechenden Primerpaare wurden so ausgewählt, dass sie die Hefe-Konsensus-Sequenz für hocheffiziente Translation (Kozak (1986) Cell 44: 283-292) neben dem Startcodon trugen. Die Amplifizierung der OtElo-DNAs wurde jeweils mit 1 µl aufgetauten Zellen, 200 µM dNTPs, 2,5 U Taq-Polymerase und 100 pmol eines jeden Primers in einem Gesamt-

volumen von 50 µl durchgeführt. Die Bedingungen für die PCR waren wie folgt: Erste Denaturierung bei 95°C für 5 Minuten, gefolgt von 30 Zyklen bei 94°C für 30 Sekunden, 55°C für 1 Minute und 72°C für 2 Minuten sowie ein letzter Verlängerungsschritt bei 72°C für 10 Minuten.

Beispiel 4: Optimierung von Elongase-Genen aus Ostreococcus tauri

Elongasen aus dem Organismus Ostreococcus tauri wurden isoliert wie in Beispiel 3 beschrieben. Um eine Steigerung des Gehalts an C22-Fettsäuren zu erreichen, wurden die Sequenzen Seq ID No. 143 (Δ6-Elongase) und Seq ID No. 109 (kodierend für ein durch die Seq ID No. 110 gekennzeichnetes Protein)(Δ5-Elongase) an die Kodonverwendung in Raps, Lein und Soja angepasst. Dazu wurde die Aminosäuresequenz der Δ6-Elongase und der Δ5-Elongase (SEQ ID NO. 144 für die Δ6-Elongase; SEQ ID NO. 65 für die Δ5-Elongase) revers translatiert, wodurch degenerierte DNA-Sequenzen erhalten wurden. Diese DNA-Sequenzen wurden mit Hilfe des Programms GeneOptimizer (Fa. Geneart, Regensburg) an die Kodonverwendung in Raps, Soja und Lein angepasst, wobei die natürliche Häufigkeit einzelner Kodons berücksichtigt wurde. Die so erhaltenen optimierten Sequenzen, die in SEQ ID NO. 64 (Δ5-Elongase) und SEQ ID NO. 122 (kodierend für ein durch die SEQ ID NO. 123 gekennzeichnetes Protein)(Δ6-Elongase) angegeben sind, wurden in vitro synthetisiert.

Beispiel 5: Klonierung von Expressionsplasmiden zur heterologen Expression in Hefen

Zur Charakterisierung der Funktion der optimierten Nukleinsäuresequenzen wurden die offenen Leserahmen der jeweiligen DNAs stromabwärts des Galactose-induzierbaren GAL1-Promotors von pYES2.1/V5-His-TOPO (Invitrogen) kloniert, wodurch die Plasmide pOTE1.2 (enthaltend die Δ6-Elongase-Sequenz) und pOTE2. 2 (enthaltend die Δ5-Elongase-Sequenz) erhalten wurden.
Übersicht zu den in den Hefe-Vektor pYES2.1/V5-His-TOPO klonierten Elongase-Sequenzen:

Gen-Name	SEQ ID	Aminosäuren

pOTE1.1, (Δ-6-Elongase)	SEQ ID NO. 143	292
pOTE1.2, (Δ-6-Elongase)	SEQ ID NO. 122	292, Kodon-optimiert
pOTE2.1, (Δ-5-Elongase)	SEQ ID NO. 109	300
pOTE2.2, (Δ-5-Elongase)	SEQ ID NO. 64	300, Kodon-optimiert

Der Saccharomyces cerevisiae-Stamm 334 wurde durch Elektroporation (1500 V) mit den Vektoren pOTE1.2 bzw. pOTE2.2 sowie den Vergleichskonstrukten pOTE1.1 und pOTE2.1, die die natürliche, für die Δ6-Elongase bzw. Δ5-Elongase kodierende Nukleinsäuresequenz enthalten, transformiert. Als Kontrolle wurde eine Hefe verwendet, die mit dem leeren Vektor pYES2 transformiert wurde. Die Selektion der transformierten Hefen erfolgte auf Komplett-Minimalmedium (CMdum)-Agarplatten mit 2% Glucose, aber ohne Uracil. Nach der Selektion wurden je drei Transformanten zur weiteren funktionellen Expression ausgewählt.

Für die Expresssion der Ot-Elongasen wurden zunächst Vorkulturen aus jeweils 5 ml CMdum-Flüssigmedium mit 2% (w/v) Raffinose aber ohne Uracil mit den ausgewählten Transformanten angeimpft und 2 Tage bei 30°C, 200 rpm inkubiert. 5 ml CMdum-Flüssigmedium (ohne Uracil) mit 2% Raffinose wurden dann mit den Vorkulturen auf eine OD_{600} von 0,05 angeimpft. Dabei wurde der Hefekultur, die mit pOTE1.1 und pOTE1.2transformiert worden war, jeweils 0,2mM γ-Linolensäure (GLA) zugegeben. Ausgehend von der Aktivität von OtELO1.1 ist eine Elongation der γ-Linolensäure zur Fettsäure 20:3 zu erwarten. Der Hefekultur, die mit pOTE2.1 und pOTE2.2 transformiert worden war, wurden jeweils 0,2mM Arachidonsäure bzw. Eicosapentaensäure zugegeben. Entsprechend der Aktivität von OtELO2.1 ist eine Elongation der Fettsäuren ARA bzw. EPA zu den Fettsäuren 22:4 bzw. 22:5 zu erwarten. Die Expression wurde durch die Zugabe von 2% (w/v) Galaktose induziert. Die Kulturen wurden für weitere 96 h bei 20°C inkubiert.

Beispiel 6: Expression von OtELO2.2 (wie in SEQ ID NO: 64 dargestellt) und OtELO1.2 (wie in SEQ ID NO: 122) in Hefen

Hefen, die wie in Beispiel 5 mit den Plasmiden pYES2, pOTE1.2, undpOTE2.1 transformiert wurden, wurden folgendermaßen analysiert:

Die Hefezellen aus den Hauptkulturen wurden durch Zentrifugation (100 x g, 5 min, 20°C) geerntet und mit 100 mM $NaHCO_3$, pH 8,0 gewaschen, um restliches Medium und Fettsäuren zu entfernen. Aus den Hefe-Zellsedimenten wurden Fettsäuremethylester (FAMEs) durch saure Methanolyse hergestellt. Hierzu wurden die Zellsedimente mit 2 ml 1 N methanolischer Schwefelsäure und 2% (v/v) Dimethoxypropan für 1 h bei 80°C inkubiert. Die Extraktion der FAMES erfolgte durch zweimalige Extraktion mit Petrolether (PE). Zur Entfernung nicht derivatisierter Fettsäuren wurden die organischen Phasen je einmal mit 2 ml 100 mM $NaHCO_3$, pH 8,0 und mit 2 ml Aqua dest. gewaschen. Anschließend wurden die PE-Phasen mit Na_2SO_4 getrocknet, unter Argon eingedampft und in 100 µl PE aufgenommen. Die Proben wurden auf einer DB-23-Kapillarsäule (30 m, 0,25 mm, 0,25 µm, Agilent) in einem Hewlett-Packard 6850-Gaschromatographen mit Flammenionisationsdetektor getrennt. Die Bedingungen für die GLC-Analyse waren wie folgt: Die Ofentemperatur wurde von 50°C bis 250°C mit einer Rate von 5°C/min und schließlich 10 min bei 250°C (Halten) programmiert.

Die Identifikation der Signale erfolgte durch Vergleiche der Retentionszeiten mit entsprechenden Fettsäurestandards (Sigma). Die Methodik ist beschrieben zum Beispiel in Napier and Michaelson (2001) Lipids 36(8):761-766; Sayanova et al. (2001) Journal of Experimental Botany 52(360):1581-1585, Sperling et al. (2001) Arch. Biochem. Biophys. 388(2):293-298 und Michaelson et al. (1998) FEBS Letters 439(3):215-218. Die Ergebnisse der Analysen sind in Tabelle 1 dargestellt.

Sowohl für pOTE1.1/pOTE1.2 als auch für pOTE2.1/2.2 konnten die entsprechenden Aktivitäten bestätigt werden. In beiden Fällen zeigte die optimierte Sequenz (pOTE1.2 bzw. pOTE2.2) Aktivität. Dabei konnte die Synthese der γ-Linolensäure durch pOTE1.2 gegenüber der Wildtyp-Sequenz nur geringfügig gesteigert werden. Dagegen konnte für

pOTE2.2 überraschenderweise sowohl eine Erhöhung der Aktivität als auch eine Veränderung der Spezifität beobachtet werden (Tabelle 1). Dabei hat sich die Aktivität zur Verlängerung von EPA nahezu verdoppelt, während die Verlängerung von ARA sich mehr als vervierfacht hat. Mit der Optimierung der Sequenz der Δ5-Elongase von Ostreococcus tauri konnte somit in Hefe bei gleicher Substratmenge die Ausbeute an den Vorstufen von DHA 6fach erhöht werden.

Beispiel 7: Klonierung von Expressionsplasmiden zur Samen-spezifischen Expression in Pflanzen

Die folgenden beschriebenen allgemeinen Bedingungen gelten für alle nachfolgenden Versuche, wenn nicht anders beschrieben.

Erfindungsgemäß bevorzugt verwendet werden für die folgenden Beispiele Bin19, pBI101, pBinAR, pGPTV, pCAMBIA oder pSUN. Eine Übersicht über binäre Vektoren und ihre Verwendung gibt Hellens et al, Trends in Plant Science (2000) 5: 446–451. Verwendet wurde ein pGPTV-Derivat wie in DE10205607 beschrieben. Dieser Vektor unterscheidet sich von pGPTV durch eine zusätzlich eingefügte AscI-Restriktionsschnittstelle.

Ausgangspunkt der Klonierung war der Klonierungsvektor pUC19 (Maniatis et al.). Im ersten Schritt wurde das Conlinin-Promotor-Fragment mit folgenden Primern amplifiziert:

Cnl1 C 5': gaattcggcgcgccgagctcctcgagcaacggttccggcggtatagagttgggtaattcga
Cnl1 C 3': cccgggatcgatgccggcagatctccaccatttttttggtggtgat

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym EcoRI und dann für 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Der Klonierungsvektor pUC19 wurde in gleicher Weise inkubiert. Anschließend wurden das PCR-Produkt und der 2668 bp große, geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1-C wurde durch Sequenzierung verifiziert.

Im nächsten Schritt wurde der OCS-Terminator (Genbank Accession V00088; De Greve, H. et al. (1982) J. Mol. Appl. Genet. 1 (6): 499-511) aus dem Vektor pGPVT-USP/OCS (DE 102 05 607) mit den folgenden Primern amplifiziert:

OCS_C 5': aggcctccatggcctgctttaatgagatatgcgagacgcc
OCS_C 3': cccgggccggacaatcagtaaattgaacggag

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym StuI und dann für 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Der Vektor pUC19-Cnl1-C wurde 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1C_OCS wurde durch Sequenzierung verifiziert.

Im nächsten Schritt wurde der Cnl1-B Promotor durch PCR mittels folgender Primer amplifiziert:

Cnl1-B 5': aggcctcaacggttccggcggtatag
Cnl1-B 3': cccggggttaacgctagcgggcccgatatcggatcccatttttggtggtgattggttct

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym StuI und dann für 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Der Vektor pUC19-Cnl1-C wurde 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1C_Cnl1B_OCS wurde durch Sequenzierung verifiziert.

In einem weiteren Schritt wurde der OCS-Terminator für Cnl1B eingefügt. Dazu wurde die PCR mit folgenden Primer durchgeführt:

OCS2 5': aggcctcctgctttaatgagatatgcgagac
OCS2 3': cccgggcggacaatcagtaaattgaacggag

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym StuI und dann für 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Der Vektor pUC19-Cnl1C_Cnl1B_OCS wurde für 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1C_Cnl1B_OCS2 wurde durch Sequenzierung verifiziert.

Im nächsten Schritt wurde der Cnl1-A Promotor durch PCR mittels folgender Primer amplifiziert:

Cnl1-B 5': aggcctcaacggttccggcggtatagag
Cnl1-B 3': aggccttctagactgcaggcggccgcccgcatttttggtggtgattggt

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde für 2 h bei 37°C mit dem Restriktionsenzym StuI inkubiert. Der Vektor pUC19-Cnl1-C wurde für 12 h bei 25°C mit dem Restriktionsenzym SmaI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 wurde durch Sequenzierung verifiziert.

In einem weiteren Schritt wurde der OCS-Terminator für Cnl1A eingefügt. Dazu wurde die PCR mit folgenden Primer durchgeführt:

OCS2 5': ggcctcctgctttaatgagatatgcga
OCS2 3': aagcttggcgcgccgagctcgtcgacggacaatcagtaaattgaacggaga

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP
1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym StuI und dann für 2 h bei 37°C mit dem Restriktionsenzym HindIII inkubiert. Der Vektor pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 wurde für 2 h bei 37°C mit dem Restriktionsenzym StuI und für 2 h bei 37°C mit dem Restriktionsenzym HindIII inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 wurde durch Sequenzierung verifiziert.

Das Plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 wurde im nächsten Schritt zur Klonierung der Δ6-, Δ5-Desaturase und Δ6-Elongase verwendet. Dazu wurde die Δ6-Desaturase aus Phytium irregulare (WO02/26946) mit folgenden PCR-Primern amplifiziert:

D6Des(Pir) 5': agatctatggtggacctcaagcctggagtg
D6Des(Pir) 3': ccatggcccgggttacatcgctgggaactcggtgat

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym BglII und dann für 2 h bei 37°C mit dem Restriktionsenzym NcoI inkubiert. Der Vektor pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 wurde für 2 h bei 37°C mit dem Restriktionsenzym BglII und für 2 h bei 37°C mit dem Restriktionsenzym NcoI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1_d6Des(Pir) wurde durch Sequenzierung verifiziert.

Das Plasmid pUC19-Cnl1_d6Des(Pir) wurde im nächsten Schritt zur Klonierung der Δ5-Desaturase aus Thraustochytrium ssp. (WO02/26946) verwendet. Dazu wurde die Δ5-Desaturase aus Thraustochytrium ssp. mit folgenden PCR-Primern amplifiziert:

D5Des(Tc) 5': gggatccatgggcaagggcagcgagggccg
D5Des(Tc) 3': ggcgccgacaccaagaagcaggactgagatatc

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$

5,00 µl 2mM dNTP
1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym BamHI und dann für 2 h bei 37°C mit dem Restriktionsenzym EcoRV inkubiert. Der Vektor pUC19-Cnl1_d6Des(Pir) wurde für 2 h bei 37°C mit dem Restriktionsenzym BamHI und für 2 h bei 37°C mit dem Restriktionsenzym EcoRV inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden den Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) wurde durch Sequenzierung verifiziert.

Das Plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) wurde im nächsten Schritt zur Klonierung der Δ6-Elongase aus Physcomitrella patens (WO01/59128) verwendet, wozu diese mit folgenden PCR-Primern amplifiziert wurde:

D6Elo(Pp) 5': gcggccgcatggaggtcgtggagagattctacggtg
D6Elo(Pp) 3': gcaaaagggagctaaaactgagtgatctaga

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP
1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55ºC
Denaturierungstemperatur: 1 min 94ºC
Elongationstemperatur: 2 min 72ºC
Anzahl der Zyklen: 35

Das PCR-Produkt wurde zuerst für 2 h bei 37ºC mit dem Restriktionsenzym NotI und dann für 2 h bei 37ºC mit dem Restriktionsenzym XbaI inkubiert. Der Vektor pUC19-CnI1_d6Des(Pir)_d5Des(Tc) wurde für 2 h bei 37ºC mit dem Restriktionsenzym NotI und für 2 h bei 37ºC mit dem Restriktionsenzym XbaI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-CnI1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurde durch Sequenzierung verifiziert.

Ausgehend von pUC19-CnI1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurde der binäre Vektor für die Pflanzentransformation hergestellt. Dazu wurde pUC19-CnI1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) für 2 h bei 37ºC mit dem Restriktionsenzym AscI inkubiert. Der Vektor pGPTV wurde in gleicher Weise behandelt. Anschließend wurden das Fragment aus pUC19-CnI1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) und der geschnittene pGPTV-Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und

PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurde durch Sequenzierung verifiziert.

Ein weiteres Konstrukt, pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co), fand Verwendung. Dazu wurde ausgehend von pUC19-Cnl1C_OCS mit folgenden Primern amplifiziert:

Cnl1_OCS 5': gtcgatcaacggttccggcggtatagagttg
Cnl1_OCS 3': gtcgatcggacaatcagtaaattgaacggaga

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP
1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde für 2 h bei 37°C mit dem Restriktionsenzym SalI inkubiert. Der Vektor pUC19 wurde für 2 h bei 37°C mit dem Restriktionsenzym SalI inkubiert. Anschließend wurden das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid

Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1_OCS wurde durch Sequenzierung verifiziert.

In einem weiteren Schritt wurde das Δ12-Desaturase-Gen aus Calendula officinalis (WO01/85968) in pUC19-Cnl1_OCS kloniert. Dazu wurde d12Des(Co) mit folgenden Primern amplifiziert:

D12Des(Co) 5': agatctatgggtgcaggcggtcgaatgc
D12Des(Co) 3': ccatggttaaatcttattacgatacc

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA
5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$
5,00 µl 2mM dNTP
1,25 µl je Primer (10 pmol/µl)
0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C
Denaturierungstemperatur: 1 min 94°C
Elongationstemperatur: 2 min 72°C
Anzahl der Zyklen: 35

Das PCR-Produkt wurde für 2 h bei 37°C mit dem Restriktionsenzym BglII und anschließend für 2 h bei gleicher Temperatur mit NcoI inkubiert. Der Vektor pUC19-Cnl1_OCS wurde in gleicher Weise inkubiert. Anschließend wurden das PCR-Fragment und der geschnittene Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-

Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1_D12Des(Co) wurde durch Sequenzierung verifiziert.

Das Plasmid pUC19-Cnl1_D12Des(Co), sowie das Plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurden für 2 h bei 37°C mit dem Restriktionsenzym SalI inkubiert. Anschließend wurde das Vektor-Fragment sowie der Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und Vektor-Fragment ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1_ d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) wurde durch Sequenzierung verifiziert.

Ausgehend von pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) wurde der binäre Vektor für die Pflanzentransformation hergestellt. Dazu wurde pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) für 2 h bei 37°C mit dem Restriktionsenzym AscI inkubiert. Der Vektor pGPTV wurde in gleicher Weise behandelt. Anschließend wurden das Fragment aus pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) und der geschnittene pGPTV-Vektor durch Agarose-Gelelektrophorese aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschließend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von Roche verwendet. Das entstandene Plasmid pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) wurde durch Sequenzierung verifiziert.

Ein weiteres Beispiel für die Verwendung von samenspezifischen Expressionskonstrukten ist der Napin Promotor. In Wu et al. (2005) Nat. Biotech. 23:1013-1017 ist die Herstellung dieser Expressionskonstrukte in den Vektoren pGPTV oder pSUN beschrieben.

Ein weiterer für die Pflanzentransformation geeigneter Vektor ist pSUN2. Um die Zahl der im Vektor enthaltenen Expressionskassetten auf mehr als vier zu erhöhen wurde dieser Vektor in Kombination mit dem Gateway-System (Invitrogen, Karlsruhe) verwendet. Dazu wurde in den Vektor pSUN2 gemäß Herstellerangaben die Gateway-Kassette A wie folgendermaßen beschrieben, eingefügt:

Der pSUN2 Vektor (1 µg) wurde 1 h mit dem Restriktionsenzym EcoRV bei 37° inkubiert. Anschließend wurde die Gateway-Kassette A (Invitrogen, Karlsruhe) in den geschnittenen Vektor ligiert mittels des Rapid Ligation Kits von Roche, Mannheim. Das entstandene Plasmid wurde in E. coli DB3.1 Zellen (Invitrogen) transformiert. Das isolierte Plasmid pSUN-GW wurde anschließend durch Sequenzierung verifiziert.

Im zweiten Schritt wurde die Expressionskassette aus pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) mittels AscI ausgeschnitten und in den in gleicherweise behandelten Vektor pSUN-GW ligiert. Das so entstandene Plasmid pSUN-4G wurde für weitere Genkonstrukte verwendet.

Dazu wurde zuerst gemäß Herstellerangaben (Invitrogen) ein pENTR-Klon modifiziert. Das Plasmid pENTR1A (Invitrogen) wurde 1 h bei 37° mit dem Restriktionsenzym EcoRI inkubiert, anschließend für 30 min mit Klenow-Enzym, sowie einem 1 µM dNTP-Mix behandelt und dann der AscI-Adapter (5'-ggcgcgcc; am 5'-Ende phosphoryliert, doppelsträngig) in den pENTR1A-Vektor liegiert. In diesen modifizierten wurde wie oben beschrieben schrittweise Gene in die Cnl-Kassette eingefügt und über AscI in den pENTR-Vektor übertragen, wodurch der pENTR-Cnl-Vektor entstand.

In einem weiteren Schritt wurde das Konstrukt pSUN-8G hergestellt. Dazu wurden 5'- und 3'-Primer für die Gene mit den SEQ ID NOs: 1, 3, 5 und 7 mit den oben beschriebenen Restriktionsschnittstellen sowie den ersten und jeweils letzten 20 Nukleotiden des offenen Leserahmens erstellt und mit den Standardbedingungen (siehe oben) amplifiziert und in den pENTR-Cnl-Vektor ligiert, der anschließend einer Rekombinationsreaktion nach Herstellerangaben mit dem Vektor pSUN-4G unterworfen wurde.

Dadurch wurde das Konstrukt pSUN-8G hergestellt, das in Brassica juncea bzw. Brassica napus transformiert wurde. Die Samen der transgenen Pflanzen wurden durch Gaschromatographie analysiert.

Ein weiteres Konstrukt, dass für die Transformation von B. juncea und B. napus verwendet wurde, war das Konstrukt pSUN-9G. Dieses Konstrukt wurde entsprechend zu Wu et al. (2005) Nat. Biotech. 23:1013-1017 mit dem Napin Promotor dargestellt. In Modifikation zu Wu et al. 2005 wurde anstelle des Genes OmELO die kodierende Sequenz von OtELO2.2 in der beschriebenen Art eingesetzt. Das erhaltene Konstrukt pSUN-9G wurde dann in B. juncea bzw. B. napus transformiert.

Beispiel 8: Lipidextraktion aus Pflanzenmaterial

Die Auswirkung der genetischen Modifikation in Pflanzen auf die Produktion einer gewünschten Verbindung (wie einer Fettsäure) kann bestimmt werden, indem die modifizierte Pflanze unter geeigneten Bedingungen (wie den vorstehend beschriebenen) gezüchtet wird und das Medium und/oder die zellulären Komponenten auf die erhöhte Produktion des gewünschten Produktes (d.h. der Lipide oder einer Fettsäure) untersucht werden. Diese Analysetechniken sind dem Fachmann bekannt und umfassen Spektroskopie, Dünnschichtchromatographie, Färbeverfahren verschiedener Art, enzymatische und mikrobiologische Verfahren sowie analytische Chromatographie, wie Hochleistungs-Flüssigkeitschromatographie (siehe beispielsweise Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon A. et al. (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A. et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; und Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

Neben den oben erwähnten Verfahren werden Pflanzenlipide aus Pflanzenmaterial wie von Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22): 12935-12940, und Browse et al. (1986) Analytic Biochemistry 152:141-145 beschrieben extrahiert. Die qualitative und quantitative Lipid- oder Fettsäureanalyse ist beschrieben bei Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

Zusätzlich zur Messung des Endproduktes der Fermentation ist es auch möglich, andere Komponenten der Stoffwechselwege zu analysieren, die zur Produktion der gewünschten Verbindung verwendet werden, wie Zwischen- und Nebenprodukte, um die Gesamteffizienz der Produktion der Verbindung zu bestimmen. Die Analyseverfahren umfassen Messungen der Nährstoffmengen im Medium (z.B. Zucker, Kohlenwasserstoffe, Stickstoffquellen, Phosphat und andere Ionen), Messungen der Biomassezusammensetzung und des Wachstums, Analyse der Produktion üblicher Metabolite von Biosynthesewegen und Messungen von Gasen, die während der Fermentation erzeugt werden. Standardverfahren für diese Messungen sind in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes und P.F. Stanbury, Hrsgb., IRL Press, S. 103-129; 131-163 und 165-192 (ISBN: 0199635773) und darin angegebenen Literaturstellen beschrieben.

Ein Beispiel ist die Analyse von Fettsäuren (Abkürzungen: FAME, Fettsäuremethylester; GC-MS, Gas-Flüssigkeitschromatographie-Massenspektrometrie; TAG, Triacylglycerin; TLC, Dünnschichtchromatographie).

Der unzweideutige Nachweis für das Vorliegen von Fettsäureprodukten kann mittels Analyse rekombinanter Organismen nach Standard-Analyseverfahren erhalten werden: GC, GC-MS oder TLC, wie verschiedentlich beschrieben von Christie und den Literaturstellen darin (1997, in: Advances on Lipid Methodology, Vierte Aufl.: Christie, Oily Press,

Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren, Lipide 33:343-353).

Das zu analysierende Material kann durch Ultraschallbehandlung, Mahlen in der Glasmühle, flüssigen Stickstoff und Mahlen oder über andere anwendbare Verfahren aufgebrochen werden. Das Material muss nach dem Aufbrechen zentrifugiert werden. Das Sediment wird in Aqua dest. resuspendiert, 10 min bei 100°C erhitzt, auf Eis abgekühlt und erneut zentrifugiert, gefolgt von Extraktion in 0,5 M Schwefelsäure in Methanol mit 2 % Dimethoxypropan für 1 Std. bei 90°C, was zu hydrolysierten Öl- und Lipidverbindungen führt, die transmethylierte Lipide ergeben. Diese Fettsäuremethylester werden in Petrolether extrahiert und schließlich einer GC-Analyse unter Verwendung einer Kapillarsäule (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 mikrom, 0,32 mm) bei einem Temperaturgradienten zwischen 170°C und 240°C für 20 min und 5 min bei 240°C unterworfen. Die Identität der erhaltenen Fettsäuremethylester muss unter Verwendung von Standards, die aus kommerziellen Quellen erhältlich sind (d.h. Sigma), definiert werden.

Pflanzenmaterial wird zunächst mechanisch durch Mörsern homogenisiert, um es einer Extraktion zugänglicher zu machen.

Dann wird 10 min auf 100°C erhitzt und nach dem Abkühlen auf Eis erneut sedimentiert. Das Zellsediment wird mit 1 M methanolischer Schwefelsäure und 2 % Dimethoxypropan für 1h bei 90°C hydrolysiert und die Lipide transmethyliert. Die resultierenden Fettsäuremethylester (FAME) werden in Petrolether extrahiert. Die extrahierten FAME werden durch Gasflüssigkeitschromatographie mit einer Kapillarsäule (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0,32 mm) und einem Temperaturgradienten von 170°C auf 240°C in 20 min und 5 min bei 240°C analysiert. Die Identität der Fettsäuremethylester wird durch Vergleich mit entsprechenden FAME-Standards (Sigma) bestätigt. Die Identität und die Position der Doppelbindung kann durch geeignete chemische Derivatisierung der FAME-Gemische z.B. zu 4,4-Dimethoxyoxazolin-Derivaten (Christie, 1998) mittels GC-MS weiter analysiert werden.

Beispiel 9: Verwendung der optimierten Δ5-Elongase (wie in SEQ ID NO: 64 dargestellt) aus Ostreococcus tauri für Konstrukte zur konstitutiven Expression

Für die Transformation von Pflanzen wurden Transformationsvektoren auf Basis von pGPTV-35S, eines Plasmids basierend auf pBIN19-35S (Bevan M. (1984) Nucl. Acids Res. 18:203) erzeugt. Dazu wurde zunächst in einem pUC-Vektor eine Expressionskassette bestehend aus dem Promotor-Element CaMV35S (Seq ID No. 161) sowie dem 35S-Terminator (SEQ ID NO. 162; Franck, A. et al. (1980) Cell 21 (1): 285-294) zusammengesetzt. Dabei wurde der Promotor über die Restriktionsschnittstellen SalI/XbaI und der Terminator über die Restriktionsschnittstellen BamHI/SmaI eingesetzt. An den Terminator wurde außerdem ein Polylinker mit der XhoI Schnittstelle angehängt (‚triple ligation'). Das entstandene Plasmid pUC19-35S wurde dann für die Klonierung von PUFA Genen eingesetzt. Es wurden parallel die offenen Leserahmen der Δ6-Desaturase- (SEQ ID NO. 1), der Δ5-Desaturase- (SEQ ID NO. 51) und Δ6-Elongase- (SEQ ID NO. 171) Sequenzen über die EcoRV-Schnittstelle in pUC19-35S Vektoren eingefügt. Die entstandenen Plasmide pUC-D6, pUC-D5, pUC-E6(Tc) wurden für die Erstellung des binären Vektors pGPTV-35S_D6D5E6(Tc) verwendet. Dazu wurde der Vektor pGPTV mit dem Enzym SalI, das Plasmid pUC-D6 mit SalI/XhoI verdaut und die korrekten Fragmente ligiert. Das entstandene Plasmid pGPTV-D6 wurde anschließend mit SalI, das Plasmid pUC-D5 mit SalI/XhoI verdaut und die korrekten Fragmente ligiert. Das entstandene Plasmid pGPTV-D6-D5 wurde dann ein weiteres Mal mit SalI verdaut, das Plasmid pUC-E6(Tc) mit SalI/XhoI und die korrekten Fragmente ligiert. Aus diesen sequentiellen Klonierungsschritten entstand der binäre Vektor pGPTV-D6D5E6(Tc), der für die Transformation eingesetzt wurde.

In einer weiteren Ausführung wurde an Stelle der Sequenz d6Elo(Tc) die Sequenz von d6Elo(Tp) (SEQ ID NO. 163) in den Vektor pUC19-35S eingesetzt. Das entstandene Plasmid pUC- E6(Tp) wurde zur Herstellung des binären Vektors pGPTV-35S_D6D5E6(Tp) verwendet.

In einer weiteren Ausführung wurde der offene Leserahmen der ω3Des(SEQ ID NO. 193) in pUC19-35S kloniert. Das entstandene Plasmid pUC-ω3Pi wurde über SalI/XhoI in

die binären Vektoren pGPTV-D6D5E6(Tc) und pGPTV-D6D5E6(Tp) übertragen. Die entstandenen Vektoren pGPTV-D6D5E6(Tc)ω3Pi und pGPTV-D6D5E6(Tp)ω3Pi wurden für die Pflanzentransformation eingesetzt.

In einer weiteren Ausführung wurde der offene Leserahmen der optimierten Δ5-Elongase aus Ostreococcus tauri (Seq ID No. 64), sowie der offene Leserahmen der Δ4-Desaturase aus Thraustochytrium sp. (Seq ID No. 77) in pUC19-35S kloniert. Die entstandenen Plasmide pUC-E5 und pUC-D4 wurden dann über SalI/XhoI entsprechend obiger Angaben in den Vektor pGPTV-D6D5E6(Tp)ω3Pi übertragen. Der entstandene Vektor pGPTV-D6D5E6(Tp)ω3PiE5D4 wurde für die Pflanzentransformation eingesetzt.

Alle binären Vektoren wurden in E. coli DH5α-Zellen (Invitrogen) nach Herstellerangaben transformiert. Positive Klone wurden durch PCR identifiziert und Plasmid-DNA isoliert (Qiagen Dneasy).

Beispiel 10: Transformation der konstitutiven binären Vektoren in Pflanzen

a) Erzeugung transgene Brassica napus bzw. Brassica juncea Pflanzen

Es wurde das Protokoll zur Transformation von Rapspflanzen verwendet (verändert nach Moloney et al. (1992) Plant Cell Reports 8:238-242).

Zur Erzeugung transgener Pflanzen wurde der binäre Vektor pGPTV-D6D5E6(Tp)ω3PiE5D4 in Agrobacterium tumefaciens C58C1:pGV2260 transformiert (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). Zur Transformation von Orychophragmus violaceus wurde eine 1:50 Verdünnung einer Übernachtkultur einer positiv transformierten Agrobakterienkolonie in Murashige-Skoog Medium (Murashige und Skoog (1962) Physiol. Plant. 15: 473) mit 3 % Saccharose (3MS-Medium) verwendet. Petiolen oder Hypokotyledonen frisch gekeimter steriler Pflanzen (zu je ca. 1 cm^2) wurden in einer Petrischale mit einer 1:50 Agrobakterienverdünnung für 5-10 Minuten inkubiert. Es folgt eine 3-tägige Koinkubation in Dunkelheit bei 25°C auf 3MS-Medium mit 0,8 % Bacto-Agar. Die Kultivierung wurde anschließend mit 16 Stunden Licht / 8 Stunden Dunkelheit und in wöchentlichem Rhythmus auf MS-Medium mit 500 mg/l Claforan (Cefotaxime-Natrium), 15 mg/l Kanamycin, 20 μM Benzylaminopurin (BAP) und 1,6 g/l Glu-

kose weitergeführt. Wachsende Sprosse wurden auf MS-Medium mit 2 % Saccharose, 250 mg/l Claforan und 0,8 % Bacto-Agar überführt. Bildeten sich nach drei Wochen keine Wurzeln, wurde dem Medium als Wachstumshormon 2-Indolbuttersäure zum Bewurzeln zugegeben.

Regenerierte Sprosse wurden auf 2MS-Medium mit Kanamycin und Claforan erhalten, nach Bewurzelung in Erde überführt und nach Kultivierung für zwei Wochen in einer Klimakammer oder im Gewächshaus angezogen, zur Blüte gebracht, reife Samen geerntet und mittels Lipidanalysen auf Elongase-Expression wie Δ-6-Elongaseaktivität oder Δ-5- oder Δ-6-Desaturaseaktivität untersucht. Linien mit erhöhten Gehalten an mehrfach ungesättigten C20- und C22-Fettsäuren wurden so identifiziert.

b) Erzeugung transgener Orychophragmus violaceus Pflanzen

Es wurde das Protokoll zur Transformation von Rapspflanzen verwendet (verändert nach Moloney et al. (1992) Plant Cell Reports 8:238-242) wie unter a) beschrieben, angewendet.

Zur Erzeugung transgener Pflanzen wurde der binäre Vektor pGPTV-D6D5E6(Tp)ω3PiE5D4 in Agrobacterium tumefaciens C58C1:pGV2260 transformiert (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). Zur Transformation von Orychophragmus violaceus wurde eine 1:50 Verdünnung einer Übernachtkultur einer positiv transformierten Agrobakterienkolonie in Murashige-Skoog Medium (Murashige und Skoog (1962) Physiol. Plant. 15: 473) mit 3 % Saccharose (3MS-Medium) verwendet. Petiolen oder Hypokotyledonen frisch gekeimter steriler Pflanzen (zu je ca. 1 cm^2) wurden in einer Petrischale mit einer 1:50 Agrobakterienverdünnung für 5-10 Minuten inkubiert. Es folgt eine 3-tägige Koinkubation in Dunkelheit bei 25°C auf 3MS-Medium mit 0,8 % Bacto-Agar. Die Kultivierung wurde anschließend mit 16 Stunden Licht / 8 Stunden Dunkelheit und in wöchentlichem Rhythmus auf MS-Medium mit 500 mg/l Claforan (Cefotaxime-Natrium), 15 mg/l Kanamycin, 20 µM Benzylaminopurin (BAP) und 1,6 g/l Glukose weitergeführt. Wachsende Sprosse wurden auf MS-Medium mit 2 % Saccharose, 250 mg/l Claforan und 0,8 % Bacto-Agar überführt. Bildeten sich nach drei Wochen kei-

ne Wurzeln, wurde dem Medium als Wachstumshormon 2-Indolbuttersäure zum Bewurzeln zugegeben.

Regenerierte Sprosse wurden auf 2MS-Medium mit Kanamycin und Claforan erhalten, nach Bewurzelung in Erde überführt und nach Kultivierung für zwei Wochen in einer Klimakammer oder im Gewächshaus angezogen, zur Blüte gebracht, reife Samen geerntet und mittels Lipidanalysen auf Elongase-Expression wie Δ-6-Elongaseaktivität oder Δ-5- oder Δ-6-Desaturaseaktivität untersucht. Linien mit erhöhten Gehalten an mehrfach ungesättigten C20- und C22-Fettsäuren wurden so identifiziert.

c) Transformation von Arabidopsis thaliana Pflanzen

Es wurde das Protokoll von Bechthold et al. (1993) C.R. Acad. Sci. Ser. III Sci. Vie. 316: 1194-1199 angewendet.

Zur Erzeugung transgener Pflanzen wurde der erzeugte binäre Vektor pGPTV-D6D5E6(Tp)ω3PiE5D4 in Agrobacterium tumefaciens C58C1:pMP90 transformiert (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788) und gemäß des Protokolls von Bechthold et al. (1993) Blüten von Arabidopsis thaliana cv. Columbia 0 in eine Agrobakterien-Lösung mit OD600=1,0 getaucht. Der Vorgang wurde zwei Tage später nochmals wiederholt. Samen aus diesen Blüten wurden dann auf Agar-Platten mit ½ MS, 2% Saccharose und 50mg/L Kanamycin ausgelegt. Grüne Keimlinge wurden dann auf Erde transferiert.

Beispiel 11: Analyse von Pflanzenmaterial transgener Orychophragmus- bzw. Arabidopsis-Pflanzen

Die Extraktion von Blattmaterial transgener Orychophragmus violaceus und Arabidopsis thaliana Pflanzen tranformiert mit pGPTV-D6D5E6(Tp)ω3PiE5D4 sowie die gaschromatographische Analyse wurde wie in Beispiel 8 beschrieben durchgeführt. Tabelle 2 zeigt die Ergebnisse der Analysen. Die verschiedenen Fettsäuren sind in Gewichtsprozent angegeben. Für beide unterschiedliche Pflanzenarten konnte die Synthese von langkettigen, mehrfach ungesättigte Fettsäuren gezeigt werden. Überraschenderweise konnte

mit der optimierten Sequenz der Δ5-Elongase (wie in SEQ ID NO: 64 dargestellt) aus Ostreococcus tauri eine deutlich höhere Ausbeute an DHA erhalten werden wie dies zum Beispiel von Robert et al. (2005) Functional Plant Biology 32:473-479 für Arabidopsis thaliana mit 1,5% DHA berichtet wird. Für Orychophragmus violaceus konnte zum ersten Mal eine Synthese von langkettigen, mehrfach ungesättigten Fettsäuren erzielt werden.

Beispiel 11: Analyse von Samen von transgener Brassica juncea Linien

Die Extraktion von Samen von transgenen Brassica juncea Pflanzen tranformiert mit pSUN-9G sowie die gaschromatographische Analyse wurde wie in Beispiel 8 beschrieben durchgeführt. Tabelle 6 zeigt die Ergebnisse der Analysen. Die verschiedenen Fettsäuren sind in Flächenprozent angegeben. Wie in Wu et al. 2005 konnte die Synthese von langkettigen mehrfachungesättigen Fettsäuren (PUFA) gezeigt werden. Überraschenderweise resultierte die Verwendung der modifizierten Elongase-Sequenz OtELO2.2 wie in der durch die SEQ ID NO: 64 beschriebenen Nukleinsäuresequenz in einer dramatischen Erhöhung des Gehalts an C22 Fettsäuren. Insgesamt enthielt das Samenöl etwa 8% Gew.-% mehrfachungsättigte C22-Fettsäuren. Im speziellen war die Fettsäure Docosahexaensäure (DHA) zu 1,9% Gew.-% im Samenöl enthalten, was einer Steigerung um Faktor 10 im Vergleich zu Wu et al. 2005 darstellte.

Beispiel 12: Detaillierte Analyse der Lipidklassen und Positionsanalyse von Blattmaterial von O. violaceus

Etwa 1 g Blattgewebe wurde für 10 Minuten bei 95°C in in 4 ml of Isopropanol erhitzt, durch Polytron homogenisiert und nach der Zugabe von 1,5 ml Chloroform geschüttelt. Die Proben wurden zentrifugiert, der Überstand gesammelt und das Pellet mit Isopropanol:Chloroform 1:1 (v/v) nochmals extrahiert. Die beiden Extrakte wurden vereinigt, getrocknet und in Chloroform gelöst. Der Lipidextrakt wurde an einer Silica PrepSep-Säule (Fisher Scientific, Nepean, Canada) in neutrale Lipide, Glykolipide und Phospholipide vorfraktioniert, wobei mit Chloroform:Essigsäure 100:1 (v/v), Aceton:Essigsäure 100:1 (v/v) bzw. Methanol:Chloroform:Wasser 100:50:40 (v/v/v) eluiert wurde. Diese Fraktionen wurden auf Silica G-25 Dünnschichtchromatographieplatten (TLC; Macherey-Nagel, Dü-

ren, Germany) weiter aufgetrennt. Neutrale Lipide wurden mit Hexan:Diethylether:Essigsäure (70:30:1), Glykolipide mit Chloroform:Methanol:Ammoniak (65:25:4 v/v/v) und Phospholipide mit Chloroform:Methanol:Ammoniak:Wasser (70:30:4:1 v/v/v/v) entwickelt. Die einzelnen Lipidklassen wurden nach dem Besprühen mit Primulin unter UV-Licht identifiziert, durch Abkratzen von den Platten entfernt, und entweder für die direkte Transmethylierung verwendet oder durch ein geeignetes Lösungsmittel für die weitere Analyse extrahiert.

Entsprechend der beschriebenen Methoden konnten die verschiedenen Lipidklassen (Neutral-, Phospho- und Galaktolipide) aufgetrennt und separat analysiert werden. Desweiteren wurden die Glycolipide auf die Position der einzelnen Fettsäuren untersucht.

a) Regiospezifische Analyse der Triacylglyceride (TAG)

Drei bis fünf mg der TLC-gereinigten TAG wurden unter Stickstoff in Glasröhrchen getrocknet, in wässrigem Puffer durch kurze Ultraschallbehandlung resuspendiert (1 M Tris pH 8; 2.2% $CaCl_2$ (w/v); 0.05% Gallensalze (w/v)) und für 4 Minuten bei 40° C inkubiert. Nach der Zugabe von 0.1 ml einer Lösung von Pankreaslipase (10 mg/ml in Wasser) wurden die Proben für 3 Minuten kräftig gevortext und der Verdau durch Zugabe von 1 ml Ethanol und 1,5 ml 4 M HCl abgebrochen. Die teilweise verdauten TAG wurden zweimal mit Diethylether extrahiert, mit Wasser gewaschen, getrocknet und in einem kleinen Volumen Chloroform gelöst. Monoacylglycerole (MAG) wurden von den freien Fettsäuren und unverdauten TAGs auf einer TLC-Platte getrennt wie oben für neutrale Lipide beschrieben. Der Punkt, der den MAGs entsprach, wurde durch GC analyert und stellte die sn-2-Position der TAG dar. Die Verteilung der Fettsäuren an den verbleibenden sn-1- and sn-3-Positionen wurde nach der folgenden Formel berechnet: sn-1 + sn-3 = (TAG x 3 – MAG)/ 2.

Diese Positionsanalyse der Triacylglyceride ergab dabei, dass EPA und DHA in den Positionen sn-2 und sn-1/3 in ähnlichen Konzentrationen vorhanden sind, während ARA insgesamt nur in geringen Mengen in den Triacylglyceriden zu finden ist und hier hauptsächlich in der Position sn-2 (Tab. 3).

b) Stereospezifische Analyse von Phospholipiden

Aufgetrenntes und extrahiertes Phosphatidylglykol (PG), Phosphatidylethanolamin (PE) und Phosphatidylcholin (PC) wurden unter N_2 getrocknet und in 0.5 ml Boratpuffer (0.5M, pH 7.5, enthaltend 0.4 mM $CaCl_2$) resuspendiert. Nach einer kurzen Ultraschallbehandlung wurden 5U der Phospholipase A2 aus dem Gift von Naja mossambica (Sigma P-7778) und 2 ml Diethylether zugegeben und die Proben wurden für 2 Stunden bei Raumtemperatur gevortext. Die Etherphase wurde getrocknet, der Verdau mit 0,3 ml 1M HCl abgebrochen, und das Reaktionsgemisch wurde mit Chloroform:Methanol (2:1 v/v) extrahiert. Die verdauten Phospholipide wurden durch TLC in Chloroform:Methanol:Ammoniak:Wasser (70:30:4:2 v/v/v/v) getrennt und Punkte, die den freigesetzten freien Fettsäuren und Lysophospholipiden entsprachen, wurden durch Kratzen entfernt und direkt transmethyliert.

Die Positionsanalyse der Phospholipide zeigte eine Akkumulation von EPA und DHA in der sn-2 Position von Phosphatidylcholin (PC), während DHA in Phosphatidylethanolamin (PE) in sn-1 und sn-2 Position ähnlich verteilt war. In beiden Phospholipiden war kein oder nur Spuren von ARA zu finden (Tab. 4). In Phosphatidylglycerol fanden sich geringere Konzentrationen an EPA und DHA als in den anderen untersuchten Phospholipiden, wobei auch in dieser Lipidklasse eine Akkumulation in der sn-2 Position zu beobachten war (Tab. 4, PG).

c) Stereospezifische Analyse von Glykolipiden

Als weitere polare Lipidklasse wurden die Galaktolipide untersucht. Galaktolipide finden sich in den Membranen von Plastiden und bilden dort die Hauptkomponenten.

TLC-gereinigtes Monogalaktosyldiacylglycerol (MGDG) und Digalaktosyldiacylglycerol (DGDG) wurden unter Stickstoff getrocknet und in 0.5 ml Diethylether gelöst. Dann wurden 25 Einheiten der Lipase aus Rhizopus arrhizus (Sigma 62305), resuspendiert in 2 ml Boratepuffer (50 mM, pH 7.5 enthaltend 2 mM $CaCl_2$), zugegeben und die Proben für 2

Stunden bei Raumtemperatur gevortext. Die Etherphase wurde getrocknet und der Verdau durch die Zugabe von 0,3 ml 1M HCl abgebrochen und die Lipide wurden mit 4 ml Chloroform:Methanol (2:1 v/v) extrahiert. Nach dem Trocknen wurden die verdauten Galaktolipide in einem kleinen Volumen Chloroform:Methanol (2:1 v/v) und zweimal auf einer vorgefertigten Silica TLC-Platte entwickelt, zuerst mit Chloroform:Methanol:Ammoniak:Wasser (70:30:4:1 v/v/v/v) bis etwa zwei Drittel der Plattenhöhe, gefolgt von der vollständigen Entwicklung in Hexan:Diethylether:Essigsäure (70:30:1). Die Punkte, die den freigesetzten freien Fettsäuren und den Lysogalaktolipiden entsprachen, wurden nach dem Besprühen mit Primulin identifiziert, abgekratzt und direkt für die GC-Analyse transmethyliert.

Auch in diesen Lipiden konnten VLCPUFA gefunden werden, wobei eine Akkumulation von EPA in der sn-2 Position zu beobachten war. DHA war nur in den Digalakto-Diacylglycerolen (DGDG) zu finden und konnte nicht in den Monogalakto-Diacylglycerolen (MGDG) nachgewiesen werden (Tabelle 5). Die Verteilung von VLCPUFA in Galaktolipiden, einem Kompartiment, in dem diese Fettsäuren nicht erwartet wurden, zeigt die Dynamik der Synthese und den späteren Umbau. Ernährungstechnisch sind VLCPUFA in Polarlipiden besonders wertvoll, da diese im Darm von Säugetieren besser aufgenommen werden können als die Neutrallipide.

Tabelle 1: Test der optimierten Sequenzen von pOTE1.1 und pOTE2.1 in Hefe. Entsprechend der Substratumsetzungen wurden die Konversionsraten bestimmt. Mit der optimierten Sequenz in Plasmid pOTE2.2 konnte ein deutlicher Anstieg der Aktivität erreicht werden.

Konversionsraten der Ostreococcus tauri Elongasen					
Genes		Substrate Product	GLA 20:3	ARA 22:4	EPA 22:5
pOTE1.1	d6Elongase(Ot)		21,1		
pOTE1.2	d6Elongase(Ot)_opt		25,6		
pOTE2.1	d5Elongase(Ot)			7,3	35,9
pOTE2.2	d5Elongase(Ot)_opt			32,7	63,1

Tabelle 2: Gaschromatographische Analyse von Blattmaterial von Orychophragmus violaceus und Arabidopsis thaliana. Die einzelnen Fettsäuren sind in Flächenprozent angegeben.

Fettsäurekomposition Blattmaterial Orychophragmus violaceus											
Fettsäuren	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Kontrolle	20,9	8,5	3,3	16,0	0,0	47,4	0,0	0,0	0,0	0,0	0,0
Trangen	21,3	8,2	5,2	5,2	4,2	23,1	5,0	0,6	13,5	2,7	4,5

Fettsäurekomposition Blattmaterial Arabidopsis thaliana											
Fettsäuren	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Kontrolle	12,8	10,0	3,5	14,2	0,0	54,6	0,0	0,0	0,0	0,0	0,0
Transgen	19,3	8,5	5,0	4,6	6,4	31,0	4,4	0,0	6,3	1,5	6,3

Tabelle 3: Regiospezifische Analyse der Triacylglyderide aus Blattmaterial von transgenen O. violaceus Pflanzen.

TAG	16:0	18:0	18:1n-9	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
wt	**25,12**	**3,03**	**5,06**		**18,53**		**44,72**							
sn-2	1,42	0,76	6,79		27,62		62,03							
sn-1+3	36,97	4,17	4,19		13,98		36,07							
Transgen	**22,63**	**3,12**	**3,46**	**0,77**	**2,35**	**9,51**	**6,37**	**13,03**	**0,74**	**0,83**	**3,87**	**24,96**	**2,22**	**4,15**
sn-2	1,62	0,64	8,33	1,61	5,15	16,21	10,88	19,84	0,17	1,38	1,99	24,82	3,27	3,02
sn-1+3	33,13	4,36	1,02	0,35	0,96	6,16	4,11	9,63	1,02	0,55	4,80	25,03	1,69	4,72

Tabelle 4: Stereospezifische Analyse der Phospholipide aus Blattmaterial von transgenen O. violaceus Pflanzen.

PG	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**27,96**	**20,04**	**4,11**	**2,89**	**0,90**		**21,82**	**0,00**	**21,56**							
sn-1	17,26	0,53	2,61	3,82	1,91		39,01	0,00	34,44							
sn-2	38,66	39,56	5,62	1,96	0,00		4,62	0,00	8,69							
Transgen	**27,15**	**24,70**	**3,08**	**4,62**	**1,20**	**0,00**	**15,15**	**1,53**	**17,94**	**1,40**	**0,00**	**0,00**	**0,45**	**2,18**	**0,10**	**0,58**
sn-1	21,16	3,61	4,23	7,52	2,14		27,40	0,50	31,57	0,81			0,38	1,24	0,00	0,33
sn-2	33,15	45,79	1,94	1,71	0,27		2,90	2,57	4,30	2,00			0,51	3,13	0,27	0,83

PE	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**37,49**	**0,00**	**6,62**	**4,35**	**1,37**		**19,28**		**29,95**							
sn-1	54,22	0,00	7,74	3,39	3,42		12,64		13,71							
sn-2	20,77	0,00	5,51	5,31	0,00		25,93		46,18							
Transgen	**31,78**	**0,81**	**5,84**	**3,08**	**2,20**	**0,85**	**5,57**	**11,25**	**11,34**	**7,38**	**0,00**	**0,00**	**2,88**	**9,41**	**1,90**	**4,90**
sn-1	50,17	0,33	10,86	3,22	4,94	0,35	2,63	3,27	3,59	2,31	0,56		4,42	6,18	0,38	4,19
sn-2	13,40	1,29	0,83	2,95	0,00	1,35	8,50	19,23	19,10	12,45	0,00		1,34	12,64	3,41	5,61

PC	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**27,67**	**0,84**	**6,38**	**8,56**	**1,80**		**21,75**		**33,01**							
sn-1	48,05	0,44	8,65	5,05	3,41		14,52		18,04							
sn-2	7,28	1,24	4,11	12,06	0,18		28,97		47,98							
Transgen	**21,00**	**0,00**	**8,01**	**10,02**	**2,86**	**1,25**	**3,77**	**11,63**	**5,60**	**12,11**	**0,50**	**0,00**	**4,34**	**11,16**	**3,76**	**3,70**
sn-1	45,35	0,00	14,71	5,08	5,70	0,31	3,23	3,09	4,58	2,65	0,61	0,08	4,01	8,32	0,41	1,18
sn-2	3,36	0,00	1,30	14,96	0,02	2,20	4,31	20,18	6,62	21,56	0,38	0,00	4,66	13,99	7,12	6,22

Tabelle 5: Stereospezifische Analyse der Galaktolipide aus Blattmaterial von transgenen O. violaceus Pflanzen.

MGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**2,64**	**0,13**	**1,23**	**30,72**	**0,33**	**0,35**	**0,26**		**3,81**		**60,52**					
sn-1	0,00	0,05	0,00	7,11	0,35	0,31	0,41		4,60		87,30					
sn-2	5,34	0,21	2,55	54,34	0,31	0,39	0,12		3,01		33,74					
tr	**4,16**	**0,20**	**1,08**	**33,81**	**0,93**	**0,73**	**0,52**	**0,03**	**1,64**	**1,88**	**44,82**	**2,73**	**0,04**	**0,30**	**0,50**	**5,08**
sn-1	1,22	0,29	0,54	4,79	1,51	1,15	0,93	0,00	2,80	0,14	80,19	0,00	0,08	0,17	0,87	3,86
sn-2	7,11	0,11	1,61	62,82	0,34	0,31	0,11	0,11	0,47	3,62	9,46	5,48	0,00	0,43	0,14	6,31

DGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**17,67**	**0,19**	**0,38**	**2,15**	**1,61**	**0,51**	**0,94**		**5,56**		**70,71**					
sn-1	16,84	0,25	0,50	2,52	2,21	0,55	1,75		6,07	0,00	68,74					
sn-2	18,50	0,12	0,27	1,78	1,01	0,46	0,13		5,05		72,68					
tr	**18,50**	**0,00**	**0,00**	**2,62**	**2,84**	**1,36**	**1,39**	**0,00**	**6,28**	**3,55**	**54,66**	**0,00**	**0,00**	**0,00**	**2,18**	**5,36**
sn-1	22,74	0,17	0,23	0,48	4,55	1,71	2,32	0,24	9,22	0,23	56,06	0,27	0,00	0,00	0,36	1,23
sn-2	14,27	0,00	0,00	4,77	1,12	1,00	0,46	0,00	3,33	6,88	53,26	0,00	0,00	0,00	4,01	9,49

Tabelle 6: Gaschromatographische Bestimmung der Fettsäuren aus Samen transgener Brassica juncea Pflanzen transformiert mit dem Konstrukt pSUN-9G in Gewichtsprozent. WT beschreibt die nicht-modifizierte Wildtyp-Kontrolle.

	Lipid Profile(%)							
	16:0	**18:0**	**18:1**	**18:2**	**γ18:3**	**α18:3**	**18:4**	**20:0**
BJ223_PUFA184_MKP71_581A	4,4	3,0	22,5	16,9	27,0	4,9	3,2	0,6
BJ223_PUFA184_MKP71_581A	4,7	3,9	17,9	10,6	29,5	4,2	4,0	0,9
BJ223_PUFA184_MKP71_581A	4,4	3,0	18,9	13,8	30,5	4,1	3,2	0,7
BJ223_PUFA184_MKP71_581A	4,6	3,3	20,5	13,2	29,8	4,2	3,3	0,8

20:3 (8,11,14)	**20:3 (11,14,17)**	**20:4 (ARA) (5,8,11,14)**	**20:4 (ETeA) (8,11,14,17)**	**20:5 (EPA) (5,8,11,14,17)**	**22:1**	**22:4**	**22:5**	**22:6**
1,1	0,5	3,1	0,6	4,8	0,0	1,5	2,0	1,5
2,0	0,9	4,2	1,0	4,1	0,0	3,1	3,5	1,9
1,3	0,7	4,1	0,5	4,5	0,0	2,7	2,8	1,8
1,4	0,6	3,8	0,6	4,4	0,0	2,4	2,5	1,8

Patentansprüche

1. Verfahren zur Herstellung von Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in einer transgenen Pflanze, umfassend das Bereitstellen in der Pflanze von mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Desaturase-Aktivität kodiert; und mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität und optional für eine Δ4-Desaturase kodiert, wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist.

2. Verfahren nach Anspruch 1, wobei die Nukleinsäuresequenz zumindest an die Kodonverwendung in Raps, Soja und/oder Lein angepasst ist.

3. Verfahren nach einem der Ansprüche 1 oder 2, wobei die Nukleinsäuresequenz unter Berücksichtigung der natürlichen Häufigkeit einzelner Kodons angepasst ist.

4. Verfahren nach einem der Ansprüche 1 bis 3, wobei die veränderte Nukleinsäuresequenz der in Seq ID No. 64 angegebenen Nukleinsäuresequenz entspricht.

5. Verfahren nach einem der Ansprüche 1 bis 4, wobei die Nukleinsäuresequenzen unter der Kontrolle eines samenspezifischen Promotors exprimiert werden.

6. Verfahren nach Anspruch 5, wobei es sich bei dem Promotor um den USP-, Vicilin-, Napin-, Glp-, SBP-, Peroxireduxin-, Legumin-, Fad3-, Conlinin- oder Oleosin-Promotor handelt.

7. Verfahren nach Anspruch 6, wobei der Gehalt an mehrfachungesättigten C22-Fettsäuren im Samenöl 5 Gew.-% oder mehr des Samenölgehalts beträgt.

8. Verfahren nach einem der vorangehenden Ansprüche, wobei zusätzlich eine oder mehrere Nukleinsäuresequenzen kodierend für ein Polypeptid mit der Aktivität einer ω3-Desaturase und/oder einer Δ4-Desaturase in der Pflanze bereitgestellt werden.

9. Verfahren nach Anspruch 8, wobei der Gehalt an Docosahexaensäure im Samenöl 1 Gew.% oder mehr des Samenölgehalts beträgt.

10. Verfahren nach einem der vorangehenden Ansprüche, wobei die Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in der Pflanze vorwiegend als Ester in Phospholipiden oder Triacylglyceriden gebunden vorliegt.

11. Verfahren nach einem der vorangehenden Ansprüche, wobei die Pflanze eine Öl-produzierende Pflanze ausgewählt aus der Gruppe bestehend aus Brassica napus, Brassica juncea und Glycine max ist.

12. Verfahren nach einem der vorangehenden Ansprüche, weiter umfassend das Gewinnen der Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in Form ihrer Öle, Lipide oder freien Fettsäuren aus der Pflanze.

.

13. Isoliertes Nukleinsäuremolekül umfassend eine Nukleinsäuresequenz gemäß Seq ID No. 64.

14. Rekombinantes Nukleinsäuremolekül, umfassend:

a) eine oder mehrere Kopien von mindestens einem in Pflanzenzellen aktiven Promotor,

b) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert,

c) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ5-Desaturase-Aktivität kodiert,

d) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert,

e) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert und die gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist, und

e) eine oder mehrere Kopien von mindestens einer Terminatorsequenz.

15. Rekombinantes Nukleinsäuremolekül nach Anspruch 14, wobei die veränderte Nukleinsäuresequenz der in Seq ID No. 64 angegebenen Nukleinsäuresequenz entspricht.

16. Rekombinantes Nukleinsäuremolekül nach einem der Ansprüche 14 oder 15, zusätzlich umfassend eine oder mehrere Nukleinsäuresequenzen kodierend für ein Polypeptid mit der Aktivität einer ω3-Desaturase und/oder einer Δ4-Desaturase.

17. Transgene Pflanze enthaltend ein rekombinantes Nukleinsäuremolekül nach einem der Ansprüche 22 bis 25 oder enthaltend die in Seq ID No. 64 angegebene Nukleinsäuresequenz.

18. Verwendung von Ölen, Lipiden oder freien Fettsäuren gemäß Anspruch 19 oder gewonnen durch ein Verfahren nach Anspruch 18 zur Herstellung von Futtermitteln, Nahrungsmitteln, Kosmetika oder Pharmazeutika.

Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren

ZUSAMMENFASSUNG

Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung von Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in transgenen Pflanzen, indem in der Pflanze bereitgestellt werden mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ6-Elongase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Desaturase-Aktivität kodiert; und mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert,
wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer Δ5-Elongase-Aktivität kodiert, gegenüber der Nukleinsäuresequenz in dem Organismus, aus dem die Sequenz stammt, dadurch verändert ist, dass sie an die Kodonverwendung in einer oder mehreren Pflanzenarten angepasst ist. Für die Herstellung von Docosahexaensäure wird zusätzlich eine oder mehrere Nukleinsäuresequenzen, die für ein Polypeptid mit einer Δ4-Desaturase-Aktivität kodiert, in die Pflanze eingebracht.

SEQUENCE LISTING

<110> BASF Plant Science GmbH

<120> Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren

<130> PF58307

<140> Current patent application

<141> 2006 09 06

<160> 199

<170> Biomax PatentTool according to PatentIN 3.1 format

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    50                  55                  60
His Pro Ser Ser Ala Leu Lys Leu Leu Glu Gln Phe Tyr Val Gly Asp
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            20                  25                  30
att att atc gac aag aag gtg tac gat gtt act gaa ttt att gaa gac       144
Ile Ile Ile Asp Lys Lys Val Tyr Asp Val Thr Glu Phe Ile Glu Asp
        35                  40                  45
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            100                 105                 110
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        115                 120                 125
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            20                  25                  30
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Ile Ile Ile Asp Lys Lys Val Tyr Asp Val Thr Glu Phe Ile Glu Asp
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cac cct ggt ggt gct caa gtt ttg ttg act cat gtt ggg aaa gat gca       192
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tct gac gta ttt cat gcc atg cat ccc gaa tct gct tac gaa gtc ttg       240
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65                  70                  75                  80
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                85                  90                  95
tct tct tct gcc caa ttc gct gta gaa atg cgt cag ctt cgg gat caa       336
Ser Ser Ser Ala Gln Phe Ala Val Glu Met Arg Gln Leu Arg Asp Gln
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        115                 120                 125
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Lys Val Leu Ser Thr Leu Ala Ile Cys Ile Ala Gly Leu Ser Leu Leu
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145                 150                 155                 160
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gga cat cat caa tgc ttc gaa gac cgc act tgg aac gat gtt ctc gtc       576
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            180                 185                 190
gta ttt ctc gga aac ttt tgt caa gga ttt tca tta tca tgg tgg aag       624
Val Phe Leu Gly Asn Phe Cys Gln Gly Phe Ser Leu Ser Trp Trp Lys
        195                 200                 205
aac aag cac aac aca cat cac gca agt aca aat gtt cat gga cag gat       672
Asn Lys His Asn Thr His His Ala Ser Thr Asn Val His Gly Gln Asp
    210                 215                 220
ccc gac att gac aca gcc cct gtt ttg ttg tgg gat gaa tat gct tct       720
Pro Asp Ile Asp Thr Ala Pro Val Leu Leu Trp Asp Glu Tyr Ala Ser
225                 230                 235                 240
gca gct tac tat gct tct ctc gat caa gaa ccc aca atg gta tct cga       768
Ala Ala Tyr Tyr Ala Ser Leu Asp Gln Glu Pro Thr Met Val Ser Arg
                245                 250                 255
ttc ctt gct gaa caa gtc ttg ccc cac caa acc cgt tat ttt ttc ttt       816
Phe Leu Ala Glu Gln Val Leu Pro His Gln Thr Arg Tyr Phe Phe Phe
            260                 265                 270
atc ctc gct ttt gct cgt ttg tct tgg gct ctt caa tct ttg agc tac       864
Ile Leu Ala Phe Ala Arg Leu Ser Trp Ala Leu Gln Ser Leu Ser Tyr
        275                 280                 285
tct ttc aag aag gag tca att aac aag tcc cgt caa ctt aac ctt ttt       912
```

```
Ser Phe Lys Lys Glu Ser Ile Asn Lys Ser Arg Gln Leu Asn Leu Phe
    290                 295                 300
gaa cgt gtt tgt att gtt ggt cac tgg gct ctc ttt gct ttt tgt atc        960
Glu Arg Val Cys Ile Val Gly His Trp Ala Leu Phe Ala Phe Cys Ile
305                 310                 315                 320
tac tct tgg tgt agc aac gtc tat cac atg gtt ttg ttt ttc ttg gtt       1008
Tyr Ser Trp Cys Ser Asn Val Tyr His Met Val Leu Phe Phe Leu Val
                325                 330                 335
agt cag gct acc aca ggc tac act tta gct ctt gtc ttt gct ttg aac       1056
Ser Gln Ala Thr Thr Gly Tyr Thr Leu Ala Leu Val Phe Ala Leu Asn
            340                 345                 350
cac aat ggt atg cct gtt atc act gaa gaa aag gct gaa tcg atg gaa       1104
His Asn Gly Met Pro Val Ile Thr Glu Glu Lys Ala Glu Ser Met Glu
        355                 360                 365
ttt ttt gag att caa gtt atc act ggt cgt gat gtt act ctt tcc ccc       1152
Phe Phe Glu Ile Gln Val Ile Thr Gly Arg Asp Val Thr Leu Ser Pro
    370                 375                 380
ttg ggt gat tgg ttc atg ggt ggt ttg aac tat caa atc gaa cat cat       1200
Leu Gly Asp Trp Phe Met Gly Gly Leu Asn Tyr Gln Ile Glu His His
385                 390                 395                 400
gtt ttc ccc aac atg cct cgt cac aac ttg cct aca gtt aag ccc atg       1248
Val Phe Pro Asn Met Pro Arg His Asn Leu Pro Thr Val Lys Pro Met
                405                 410                 415
gtg aaa tcg ctt tgt cag aaa tat gac att aac tat cac gat act gga       1296
Val Lys Ser Leu Cys Gln Lys Tyr Asp Ile Asn Tyr His Asp Thr Gly
            420                 425                 430
ttc tta aag gga aca tta gaa gta tta caa aca ctg gat att aca tcc       1344
Phe Leu Lys Gly Thr Leu Glu Val Leu Gln Thr Leu Asp Ile Thr Ser
        435                 440                 445
aag ctc tct ttg caa tta agc aaa aag tcg ttt taa                      1380
Lys Leu Ser Leu Gln Leu Ser Lys Lys Ser Phe
    450                 455
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<210> 6
<211> 459
<212> PRT
<213> Rhizopus stolonifer

<400> 6

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Met Ser Thr Leu Asp Arg Gln Ser Ile Phe Thr Ile Lys Glu Leu Glu
1               5                  10                  15
Ser Ile Ser Gln Arg Ile His Asp Gly Asp Glu Glu Ala Met Lys Phe
            20                  25                  30
Ile Ile Ile Asp Lys Lys Val Tyr Asp Val Thr Glu Phe Ile Glu Asp
        35                  40                  45
His Pro Gly Gly Ala Gln Val Leu Leu Thr His Val Gly Lys Asp Ala
    50                  55                  60
Ser Asp Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Val Leu
65                  70                  75                  80
Asn Asn Tyr Phe Val Gly Asp Val Gln Glu Thr Val Val Thr Glu Lys
                85                  90                  95
Ser Ser Ser Ala Gln Phe Ala Val Glu Met Arg Gln Leu Arg Asp Gln
            100                 105                 110
Leu Lys Lys Glu Gly Tyr Phe His Ser Ser Lys Leu Phe Tyr Ala Tyr
        115                 120                 125
Lys Val Leu Ser Thr Leu Ala Ile Cys Ile Ala Gly Leu Ser Leu Leu
    130                 135                 140
Tyr Ala Tyr Gly Arg Thr Ser Thr Leu Ala Val Val Ala Ser Ala Ile
145                 150                 155                 160
Thr Val Gly Ile Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe
                165                 170                 175
Gly His His Gln Cys Phe Glu Asp Arg Thr Trp Asn Asp Val Leu Val
            180                 185                 190
Val Phe Leu Gly Asn Phe Cys Gln Gly Phe Ser Leu Ser Trp Trp Lys
        195                 200                 205
Asn Lys His Asn Thr His His Ala Ser Thr Asn Val His Gly Gln Asp
    210                 215                 220
Pro Asp Ile Asp Thr Ala Pro Val Leu Leu Trp Asp Glu Tyr Ala Ser
```

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225                 230                 235                 240
Ala Ala Tyr Tyr Ala Ser Leu Asp Gln Glu Pro Thr Met Val Ser Arg
                245                 250                 255
Phe Leu Ala Glu Gln Val Leu Pro His Gln Thr Arg Tyr Phe Phe Phe
            260                 265                 270
Ile Leu Ala Phe Ala Arg Leu Ser Trp Ala Leu Gln Ser Leu Ser Tyr
        275                 280                 285
Ser Phe Lys Lys Glu Ser Ile Asn Lys Ser Arg Gln Leu Asn Leu Phe
    290                 295                 300
Glu Arg Val Cys Ile Val Gly His Trp Ala Leu Phe Ala Phe Cys Ile
305                 310                 315                 320
Tyr Ser Trp Cys Ser Asn Val Tyr His Met Val Leu Phe Phe Leu Val
                325                 330                 335
Ser Gln Ala Thr Thr Gly Tyr Thr Leu Ala Leu Val Phe Ala Leu Asn
            340                 345                 350
His Asn Gly Met Pro Val Ile Thr Glu Glu Lys Ala Glu Ser Met Glu
        355                 360                 365
Phe Phe Glu Ile Gln Val Ile Thr Gly Arg Asp Val Thr Leu Ser Pro
    370                 375                 380
Leu Gly Asp Trp Phe Met Gly Gly Leu Asn Tyr Gln Ile Glu His His
385                 390                 395                 400
Val Phe Pro Asn Met Pro Arg His Asn Leu Pro Thr Val Lys Pro Met
                405                 410                 415
Val Lys Ser Leu Cys Gln Lys Tyr Asp Ile Asn Tyr His Asp Thr Gly
            420                 425                 430
Phe Leu Lys Gly Thr Leu Glu Val Leu Gln Thr Leu Asp Ile Thr Ser
        435                 440                 445
Lys Leu Ser Leu Gln Leu Ser Lys Lys Ser Phe
    450                 455

<210> 7
<211> 1401
<212> DNA
<213> Cunninghamella echinulata

<220>
<221> CDS
<222> (1)..(1401)

<400> 7
atg tca ggg caa act cga gtt ttt aag cga tcc gaa gtg tcc gat tcg        48
Met Ser Gly Gln Thr Arg Val Phe Lys Arg Ser Glu Val Ser Asp Ser
1               5                   10                  15
ctc aag gcg tac caa gcg ggc gac aag aac gcc gac aag ttc ctg att        96
Leu Lys Ala Tyr Gln Ala Gly Asp Lys Asn Ala Asp Lys Phe Leu Ile
            20                  25                  30
gtc gac aac aag gtg tac gac atc acg gat ttc att gct gat cat cct       144
Val Asp Asn Lys Val Tyr Asp Ile Thr Asp Phe Ile Ala Asp His Pro
        35                  40                  45
ggc ggt gct cag gtc atc tct acg cac att ggc aag gat gcc tct gat       192
Gly Gly Ala Gln Val Ile Ser Thr His Ile Gly Lys Asp Ala Ser Asp
    50                  55                  60
gtg ttt cat gcg atg cat ccc gag tct gcg tac gaa ttg ctt gcg aat       240
Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Leu Leu Ala Asn
65                  70                  75                  80
tgc tac gtt ggc gat ctt gct gcg gac cat gct ggt gtg cag ggc gaa       288
Cys Tyr Val Gly Asp Leu Ala Ala Asp His Ala Gly Val Gln Gly Glu
                85                  90                  95
ttg gtg aat ggt gta cac aaa aag tcc aag gca ttt gcg gac gag atg       336
Leu Val Asn Gly Val His Lys Lys Ser Lys Ala Phe Ala Asp Glu Met
            100                 105                 110
cga tcg ttg cgc gag cgt ctt gag acg gag ggt gcc ttt aat ggc agt       384
Arg Ser Leu Arg Glu Arg Leu Glu Thr Glu Gly Ala Phe Asn Gly Ser
        115                 120                 125
gtt ccg ttt tac att tac aag gtg gtg tcg acg ttg gcc att ggt gcg       432
Val Pro Phe Tyr Ile Tyr Lys Val Val Ser Thr Leu Ala Ile Gly Ala
    130                 135                 140
acg ggt ctg gcc atg ctg tac tat ggc ggc cat tcg acg tcg gtc gtg       480
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Thr Gly Leu Ala Met Leu Tyr Tyr Gly Gly His Ser Thr Ser Val Val
145                 150                 155                 160
ctt gcg gcg gcg gtg gtt gtc ggt ctg ttt tgg caa cag tgc ggc tgg        528
Leu Ala Ala Ala Val Val Val Gly Leu Phe Trp Gln Gln Cys Gly Trp
                165                 170                 175
ctc gcc cac gac ttt ggc cac cac cag gcg ttt gcg gac cat acg gtc        576
Leu Ala His Asp Phe Gly His His Gln Ala Phe Ala Asp His Thr Val
            180                 185                 190
aat gac gtg atg att gcg ttt ttg ggt gga ttc tgc caa ggt ttc tcg        624
Asn Asp Val Met Ile Ala Phe Leu Gly Gly Phe Cys Gln Gly Phe Ser
        195                 200                 205
ctg tcg tgg tgg aag aac aag cac aac act cac cac gcc tcc acc aac        672
Leu Ser Trp Trp Lys Asn Lys His Asn Thr His His Ala Ser Thr Asn
    210                 215                 220
gtc cac ggc cac gat ccc gat atc gat acg gcc cct gtc ctc ctc tgg        720
Val His Gly His Asp Pro Asp Ile Asp Thr Ala Pro Val Leu Leu Trp
225                 230                 235                 240
gac gaa ttt gcc acc gcc aat ttc tat gga aac ctc gag ggt caa aag        768
Asp Glu Phe Ala Thr Ala Asn Phe Tyr Gly Asn Leu Glu Gly Gln Lys
                245                 250                 255
gat tcg gcg ttc tcg cgc ttc att gcc gaa cac gtg ctc ccc tac cag        816
Asp Ser Ala Phe Ser Arg Phe Ile Ala Glu His Val Leu Pro Tyr Gln
            260                 265                 270
acg cgc tat tac ttt ttt gtc ctc ggc ttt gcg cgt ctc tcg tgg gcc        864
Thr Arg Tyr Tyr Phe Phe Val Leu Gly Phe Ala Arg Leu Ser Trp Ala
        275                 280                 285
atc caa tcg ctc caa tac tcg ttc act gtc ggc aca ctc aac aag tca        912
Ile Gln Ser Leu Gln Tyr Ser Phe Thr Val Gly Thr Leu Asn Lys Ser
    290                 295                 300
aag acc ctc aat ctg ttt gag cgc acc atg ctt gtc agc cat tgg atc        960
Lys Thr Leu Asn Leu Phe Glu Arg Thr Met Leu Val Ser His Trp Ile
305                 310                 315                 320
ttg ttc acc act tgg acg ctc ctc ttc atc aat tca tgg acc aac atg       1008
Leu Phe Thr Thr Trp Thr Leu Leu Phe Ile Asn Ser Trp Thr Asn Met
                325                 330                 335
gtc atg ttc ttt gtc gtc agc cag gcc acc acc ggc tac gcg ctt gcc       1056
Val Met Phe Phe Val Val Ser Gln Ala Thr Thr Gly Tyr Ala Leu Ala
            340                 345                 350
ctc gtt ttc gcc atg aac cac tcg ggc atg ccc gtc ctc acc caa gaa       1104
Leu Val Phe Ala Met Asn His Ser Gly Met Pro Val Leu Thr Gln Glu
        355                 360                 365
cag gcc caa aag atg gag ttc tac gag atc cag gtc gtc acc gcc cgc       1152
Gln Ala Gln Lys Met Glu Phe Tyr Glu Ile Gln Val Val Thr Ala Arg
    370                 375                 380
gac gtc acc ctc ggt gcc ctt ggc gac tgg ttc tgc ggc ggc ctc aac       1200
Asp Val Thr Leu Gly Ala Leu Gly Asp Trp Phe Cys Gly Gly Leu Asn
385                 390                 395                 400
tat caa atc gag cac cat gta ttc cct gat atg ccc cgc cac tac ctg       1248
Tyr Gln Ile Glu His His Val Phe Pro Asp Met Pro Arg His Tyr Leu
                405                 410                 415
ccc aag gtc aag cct caa gtc aag gct ctc tgc aaa aaa cac aac att       1296
Pro Lys Val Lys Pro Gln Val Lys Ala Leu Cys Lys Lys His Asn Ile
            420                 425                 430
ttg tac cat gat acc tct gct ttg cgc ggc acc ttg gaa gtg ctg caa       1344
Leu Tyr His Asp Thr Ser Ala Leu Arg Gly Thr Leu Glu Val Leu Gln
        435                 440                 445
acc ttg gat gtt gtc caa aag ctc tgc gca agt tct ctc aaa aga tgt       1392
Thr Leu Asp Val Val Gln Lys Leu Cys Ala Ser Ser Leu Lys Arg Cys
    450                 455                 460
ttt aga tga                                                          1401
Phe Arg
465
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<210> 8
<211> 466
<212> PRT
<213> Cunninghamella echinulata

<400> 8

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Met Ser Gly Gln Thr Arg Val Phe Lys Arg Ser Glu Val Ser Asp Ser
1               5                  10                  15
Leu Lys Ala Tyr Gln Ala Gly Asp Lys Asn Ala Asp Lys Phe Leu Ile
            20                  25                  30
Val Asp Asn Lys Val Tyr Asp Ile Thr Asp Phe Ile Ala Asp His Pro
        35                  40                  45
Gly Gly Ala Gln Val Ile Ser Thr His Ile Gly Lys Asp Ala Ser Asp
    50                  55                  60
Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Leu Leu Ala Asn
65                  70                  75                  80
Cys Tyr Val Gly Asp Leu Ala Ala Asp His Ala Gly Val Gln Gly Glu
                85                  90                  95
Leu Val Asn Gly Val His Lys Lys Ser Lys Ala Phe Ala Asp Glu Met
            100                 105                 110
Arg Ser Leu Arg Glu Arg Leu Glu Thr Glu Gly Ala Phe Asn Gly Ser
        115                 120                 125
Val Pro Phe Tyr Ile Tyr Lys Val Val Ser Thr Leu Ala Ile Gly Ala
    130                 135                 140
Thr Gly Leu Ala Met Leu Tyr Tyr Gly Gly His Ser Thr Ser Val Val
145                 150                 155                 160
Leu Ala Ala Ala Val Val Val Gly Leu Phe Trp Gln Gln Cys Gly Trp
                165                 170                 175
Leu Ala His Asp Phe Gly His His Gln Ala Phe Ala Asp His Thr Val
            180                 185                 190
Asn Asp Val Met Ile Ala Phe Leu Gly Gly Phe Cys Gln Gly Phe Ser
        195                 200                 205
Leu Ser Trp Trp Lys Asn Lys His Asn Thr His His Ala Ser Thr Asn
    210                 215                 220
Val His Gly His Asp Pro Asp Ile Asp Thr Ala Pro Val Leu Leu Trp
225                 230                 235                 240
Asp Glu Phe Ala Thr Ala Asn Phe Tyr Gly Asn Leu Glu Gly Gln Lys
                245                 250                 255
Asp Ser Ala Phe Ser Arg Phe Ile Ala Glu His Val Leu Pro Tyr Gln
            260                 265                 270
Thr Arg Tyr Tyr Phe Phe Val Leu Gly Phe Ala Arg Leu Ser Trp Ala
        275                 280                 285
Ile Gln Ser Leu Gln Tyr Ser Phe Thr Val Gly Thr Leu Asn Lys Ser
    290                 295                 300
Lys Thr Leu Asn Leu Phe Glu Arg Thr Met Leu Val Ser His Trp Ile
305                 310                 315                 320
Leu Phe Thr Thr Trp Thr Leu Leu Phe Ile Asn Ser Trp Thr Asn Met
                325                 330                 335
Val Met Phe Phe Val Val Ser Gln Ala Thr Thr Gly Tyr Ala Leu Ala
            340                 345                 350
Leu Val Phe Ala Met Asn His Ser Gly Met Pro Val Leu Thr Gln Glu
        355                 360                 365
Gln Ala Gln Lys Met Glu Phe Tyr Glu Ile Gln Val Val Thr Ala Arg
    370                 375                 380
Asp Val Thr Leu Gly Ala Leu Gly Asp Trp Phe Cys Gly Gly Leu Asn
385                 390                 395                 400
Tyr Gln Ile Glu His His Val Phe Pro Asp Met Pro Arg His Tyr Leu
                405                 410                 415
Pro Lys Val Lys Pro Gln Val Lys Ala Leu Cys Lys Lys His Asn Ile
            420                 425                 430
Leu Tyr His Asp Thr Ser Ala Leu Arg Gly Thr Leu Glu Val Leu Gln
        435                 440                 445
Thr Leu Asp Val Val Gln Lys Leu Cys Ala Ser Ser Leu Lys Arg Cys
    450                 455                 460
Phe Arg
465
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<210> 9
<211> 1398
<212> DNA
<213> Glossomastix chrysoplasta

<220>

<221> CDS
<222> (1)..(1398)

<400> 9

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atg ggt aaa gga ggc cgg gac gcc ggc gct gtc ggc ggc gaa gcg gag          48
Met Gly Lys Gly Gly Arg Asp Ala Gly Ala Val Gly Gly Glu Ala Glu
1               5                  10                  15
aag acg ctg ccc aag ttt acg ttg gag gag atc cag aag cat cgc acg          96
Lys Thr Leu Pro Lys Phe Thr Leu Glu Glu Ile Gln Lys His Arg Thr
            20                  25                  30
ccg cag gat gcg tgg atg gtg cac cac aac aag gtg tac gat gtg tcc         144
Pro Gln Asp Ala Trp Met Val His His Asn Lys Val Tyr Asp Val Ser
        35                  40                  45
aac tat atg gac cac ccc ggc ggt ctg gtg atc ttt agc cac gcg ggc         192
Asn Tyr Met Asp His Pro Gly Gly Leu Val Ile Phe Ser His Ala Gly
    50                  55                  60
gat gac atg acc gac gtc ttc gcg gcg ttc cac ccg ccc tcg gcg ttc         240
Asp Asp Met Thr Asp Val Phe Ala Ala Phe His Pro Pro Ser Ala Phe
65                  70                  75                  80
aac ttc atg gac aag ttc ttg att ggc gtg gtg gac agc aag ggc tcg         288
Asn Phe Met Asp Lys Phe Leu Ile Gly Val Val Asp Ser Lys Gly Ser
                85                  90                  95
tcg ccg cag ctc cag aag gac gcg tcc cag gcc agc ttc gag aag gca         336
Ser Pro Gln Leu Gln Lys Asp Ala Ser Gln Ala Ser Phe Glu Lys Ala
            100                 105                 110
tac cgc aat ctg cga gtc cag ctc aag aag gcc ggc atg ttc aag gcc         384
Tyr Arg Asn Leu Arg Val Gln Leu Lys Lys Ala Gly Met Phe Lys Ala
        115                 120                 125
tcg agc ctc ttc tac acc tac aag gtg ctc tcg acc ctg gcg ctg tgc         432
Ser Ser Leu Phe Tyr Thr Tyr Lys Val Leu Ser Thr Leu Ala Leu Cys
    130                 135                 140
ctc gtg tcc tgg ggc ctc gtg ctg ggg tcg gac cac ttc ggt gtg cac         480
Leu Val Ser Trp Gly Leu Val Leu Gly Ser Asp His Phe Gly Val His
145                 150                 155                 160
ctc gtg ggc gct ctc ttc ctc gcg ctc ttc tgg cag cag tgc ggc tgg         528
Leu Val Gly Ala Leu Phe Leu Ala Leu Phe Trp Gln Gln Cys Gly Trp
                165                 170                 175
ttg gcc cac gac ttc ctg cac cac cag gtc ttc cag aac cgg gct cac         576
Leu Ala His Asp Phe Leu His His Gln Val Phe Gln Asn Arg Ala His
            180                 185                 190
ggc gac ctc gcc ggt atc atg atc ggc aac gtg tgg cag ggc ttc tcc         624
Gly Asp Leu Ala Gly Ile Met Ile Gly Asn Val Trp Gln Gly Phe Ser
        195                 200                 205
gtg gcc tgg tgg aag aac aag cac aac acc cac cac tcg gtg ccg aac         672
Val Ala Trp Trp Lys Asn Lys His Asn Thr His His Ser Val Pro Asn
    210                 215                 220
ctc tac gag tcg cag ccc gac gcc gcc gac ggc gat ccg gac atc gac         720
Leu Tyr Glu Ser Gln Pro Asp Ala Ala Asp Gly Asp Pro Asp Ile Asp
225                 230                 235                 240
acc atg ccc ctc ctc gcc tgg tcg ctg cgc atg gcc aag aac gcg gac         768
Thr Met Pro Leu Leu Ala Trp Ser Leu Arg Met Ala Lys Asn Ala Asp
                245                 250                 255
aac gcg cta tcc cgg tgg ttc gtg tcc cac cag gcc ttc tgc tac ttc         816
Asn Ala Leu Ser Arg Trp Phe Val Ser His Gln Ala Phe Cys Tyr Phe
            260                 265                 270
ccc atc ctt ggc ctc gcg cgg ctc tcg tgg ctc gag ggg tcc ttc tcc         864
Pro Ile Leu Gly Leu Ala Arg Leu Ser Trp Leu Glu Gly Ser Phe Ser
        275                 280                 285
ttc gtg ttc tcc aac ccg ttg gca tgg aag acg aag aac ctt gat gta         912
Phe Val Phe Ser Asn Pro Leu Ala Trp Lys Thr Lys Asn Leu Asp Val
    290                 295                 300
gcc aag cag ctc gtg acc aac ccc ctg ttg gag cag gcc ggc ctc ctg         960
Ala Lys Gln Leu Val Thr Asn Pro Leu Leu Glu Gln Ala Gly Leu Leu
305                 310                 315                 320
gtc cac tac gcc tgg gtc ttc gcc ctc tgc gcg tgc acg ggc tcc ctg        1008
Val His Tyr Ala Trp Val Phe Ala Leu Cys Ala Cys Thr Gly Ser Leu
                325                 330                 335
gtg cgc gcc ctg gcg ttc ttc ttc gtg gcc acc tgc acc agc ggc ctt        1056
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Val Arg Ala Leu Ala Phe Phe Phe Val Ala Thr Cys Thr Ser Gly Leu
                340                 345                 350
ctc ctc gcc atc gtc ttc ggc ctc ggc cac aac ggc atg gcg ctc tac      1104
Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met Ala Leu Tyr
            355                 360                 365
gag gcc aat gcc cgg ccg gac ttc tgg aag ctc cag gtg acc acc acg      1152
Glu Ala Asn Ala Arg Pro Asp Phe Trp Lys Leu Gln Val Thr Thr Thr
        370                 375                 380
cgg aac atc acc ggc agc ccc ttt gtg cac tgg ttc tgc ggc ggc ctc      1200
Arg Asn Ile Thr Gly Ser Pro Phe Val His Trp Phe Cys Gly Gly Leu
385                 390                 395                 400
cag ttc cag gtg gag cac cac ctc ttc ccc tcg ctg ccc cgg cac aac      1248
Gln Phe Gln Val Glu His His Leu Phe Pro Ser Leu Pro Arg His Asn
                405                 410                 415
ctc ccc cgg gcc cac gag atc gtg acg gcc ttc tgc aag gag cag ggc      1296
Leu Pro Arg Ala His Glu Ile Val Thr Ala Phe Cys Lys Glu Gln Gly
            420                 425                 430
gtc aag tac cac gag gcc gac ctc ctt acc ggg acc aag gag att ctt      1344
Val Lys Tyr His Glu Ala Asp Leu Leu Thr Gly Thr Lys Glu Ile Leu
        435                 440                 445
tcg tgc ttg tcg gag gta acg acg gag ttc ctc gac gag ttc cca gcc      1392
Ser Cys Leu Ser Glu Val Thr Thr Glu Phe Leu Asp Glu Phe Pro Ala
    450                 455                 460
atg taa                                                             1398
Met
465

<210> 10
<211> 465
<212> PRT
<213> Glossomastix chrysoplasta

<400> 10
Met Gly Lys Gly Gly Arg Asp Ala Gly Ala Val Gly Gly Glu Ala Glu
1               5                  10                  15
Lys Thr Leu Pro Lys Phe Thr Leu Glu Glu Ile Gln Lys His Arg Thr
            20                  25                  30
Pro Gln Asp Ala Trp Met Val His His Asn Lys Val Tyr Asp Val Ser
        35                  40                  45
Asn Tyr Met Asp His Pro Gly Gly Leu Val Ile Phe Ser His Ala Gly
    50                  55                  60
Asp Asp Met Thr Asp Val Phe Ala Ala Phe His Pro Pro Ser Ala Phe
65                  70                  75                  80
Asn Phe Met Asp Lys Phe Leu Ile Gly Val Val Asp Ser Lys Gly Ser
                85                  90                  95
Ser Pro Gln Leu Gln Lys Asp Ala Ser Gln Ala Ser Phe Glu Lys Ala
            100                 105                 110
Tyr Arg Asn Leu Arg Val Gln Leu Lys Lys Ala Gly Met Phe Lys Ala
        115                 120                 125
Ser Ser Leu Phe Tyr Thr Tyr Lys Val Leu Ser Thr Leu Ala Leu Cys
    130                 135                 140
Leu Val Ser Trp Gly Leu Val Leu Gly Ser Asp His Phe Gly Val His
145                 150                 155                 160
Leu Val Gly Ala Leu Phe Leu Ala Leu Phe Trp Gln Gln Cys Gly Trp
                165                 170                 175
Leu Ala His Asp Phe Leu His His Gln Val Phe Gln Asn Arg Ala His
            180                 185                 190
Gly Asp Leu Ala Gly Ile Met Ile Gly Asn Val Trp Gln Gly Phe Ser
        195                 200                 205
Val Ala Trp Trp Lys Asn Lys His Asn Thr His His Ser Val Pro Asn
    210                 215                 220
Leu Tyr Glu Ser Gln Pro Asp Ala Ala Asp Gly Asp Pro Asp Ile Asp
225                 230                 235                 240
Thr Met Pro Leu Leu Ala Trp Ser Leu Arg Met Ala Lys Asn Ala Asp
                245                 250                 255
Asn Ala Leu Ser Arg Trp Phe Val Ser His Gln Ala Phe Cys Tyr Phe
            260                 265                 270
Pro Ile Leu Gly Leu Ala Arg Leu Ser Trp Leu Glu Gly Ser Phe Ser
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          275                 280                 285
Phe Val  Phe Ser  Asn Pro  Leu Ala  Trp Lys  Thr Lys  Asn Leu  Asp Val
     290                 295                 300
Ala Lys  Gln Leu  Val Thr  Asn Pro  Leu Leu  Glu Gln  Ala Gly  Leu Leu
305                 310                 315                 320
Val His  Tyr Ala  Trp Val  Phe Ala  Leu Cys  Ala Cys  Thr Gly  Ser Leu
                325                 330                 335
Val Arg  Ala Leu  Ala Phe  Phe Phe  Val Ala  Thr Cys  Thr Ser  Gly Leu
            340                 345                 350
Leu Leu  Ala Ile  Val Phe  Gly Leu  Gly His  Asn Gly  Met Ala  Leu Tyr
          355                 360                 365
Glu Ala  Asn Ala  Arg Pro  Asp Phe  Trp Lys  Leu Gln  Val Thr  Thr Thr
     370                 375                 380
Arg Asn  Ile Thr  Gly Ser  Pro Phe  Val His  Trp Phe  Cys Gly  Gly Leu
385                 390                 395                 400
Gln Phe  Gln Val  Glu His  His Leu  Phe Pro  Ser Leu  Pro Arg  His Asn
                405                 410                 415
Leu Pro  Arg Ala  His Glu  Ile Val  Thr Ala  Phe Cys  Lys Glu  Gln Gly
            420                 425                 430
Val Lys  Tyr His  Glu Ala  Asp Leu  Leu Thr  Gly Thr  Lys Glu  Ile Leu
          435                 440                 445
Ser Cys  Leu Ser  Glu Val  Thr Thr  Glu Phe  Leu Asp  Glu Phe  Pro Ala
     450                 455                 460
Met
465

<210> 11
<211> 1455
<212> DNA
<213> Thalassiosira pseudonana

<220>
<221> CDS
<222> (1)..(1455)

<400> 11
atg gga aaa gga gga gac gca gcc gca gct acc aag cgt agt gga gca        48
Met Gly Lys Gly Gly Asp Ala Ala Ala Ala Thr Lys Arg Ser Gly Ala
1               5                  10                  15
ttg aaa ttg gcg gag aag ccg cag aag tac act tgg cag gag gtg aag        96
Leu Lys Leu Ala Glu Lys Pro Gln Lys Tyr Thr Trp Gln Glu Val Lys
            20                  25                  30
aag cac atc acc ccc gac gat gcc tgg gta gtc cac caa aac aaa gtc       144
Lys His Ile Thr Pro Asp Asp Ala Trp Val Val His Gln Asn Lys Val
        35                  40                  45
tac gac gtc tcc aac tgg tac gac cac ccc ggt gga gcc gtg gtg ttc       192
Tyr Asp Val Ser Asn Trp Tyr Asp His Pro Gly Gly Ala Val Val Phe
    50                  55                  60
acc cac gcc gga gac gac atg acg gac atc ttc gcc gcc ttc cac gcc       240
Thr His Ala Gly Asp Asp Met Thr Asp Ile Phe Ala Ala Phe His Ala
65                  70                  75                  80
caa ggc tct cag gcc atg atg aag aag ttt tac att gga gat ttg att       288
Gln Gly Ser Gln Ala Met Met Lys Lys Phe Tyr Ile Gly Asp Leu Ile
                85                  90                  95
ccg gag agt gtg gag cat aag gat caa aga cag ttg gat ttc gag aag       336
Pro Glu Ser Val Glu His Lys Asp Gln Arg Gln Leu Asp Phe Glu Lys
            100                 105                 110
gga tat cgt gat tta cgg gcc aag ctt gtc atg atg ggg atg ttc aag       384
Gly Tyr Arg Asp Leu Arg Ala Lys Leu Val Met Met Gly Met Phe Lys
        115                 120                 125
tcg agt aag atg tat tat gca tac aag tgc tcg ttc aat atg tgc atg       432
Ser Ser Lys Met Tyr Tyr Ala Tyr Lys Cys Ser Phe Asn Met Cys Met
    130                 135                 140
tgg ttg gtg gcg gtg gcc atg gtg tac tac tcg gac agt ttg gca atg       480
Trp Leu Val Ala Val Ala Met Val Tyr Tyr Ser Asp Ser Leu Ala Met
145                 150                 155                 160
cac att gga tcg gct ctc ttg ttg gga ttg ttc tgg cag cag tgt gga       528
His Ile Gly Ser Ala Leu Leu Leu Gly Leu Phe Trp Gln Gln Cys Gly
```

```
                    165                 170                 175
tgg ctt gcg cac gac ttt ctt cac cac caa gtc ttt aag caa cga aag        576
Trp Leu Ala His Asp Phe Leu His His Gln Val Phe Lys Gln Arg Lys
                180                 185                 190
tac gga gat ctc gtt ggc atc ttt tgg gga gat ctc atg cag ggg ttc        624
Tyr Gly Asp Leu Val Gly Ile Phe Trp Gly Asp Leu Met Gln Gly Phe
            195                 200                 205
tcg atg cag tgg tgg aag aac aag cac aat ggc cac cat gct gtt ccc        672
Ser Met Gln Trp Trp Lys Asn Lys His Asn Gly His His Ala Val Pro
        210                 215                 220
aac ttg cac aac tct tcc ttg gac agt cag gat ggt gat ccc gat att        720
Asn Leu His Asn Ser Ser Leu Asp Ser Gln Asp Gly Asp Pro Asp Ile
225                 230                 235                 240
gat acc atg cca ctc ctt gct tgg agt ctc aag cag gct cag agt ttc        768
Asp Thr Met Pro Leu Leu Ala Trp Ser Leu Lys Gln Ala Gln Ser Phe
                245                 250                 255
aga gag atc aat aag gga aag gac agt acc ttc gtc aag tac gct atc        816
Arg Glu Ile Asn Lys Gly Lys Asp Ser Thr Phe Val Lys Tyr Ala Ile
            260                 265                 270
aaa ttc cag gca ttc aca tac ttc ccc atc ctc ctc ttg gct cgc atc        864
Lys Phe Gln Ala Phe Thr Tyr Phe Pro Ile Leu Leu Leu Ala Arg Ile
        275                 280                 285
tct tgg ttg aat gaa tcc ttc aaa act gca ttc gga ctc gga gct gcc        912
Ser Trp Leu Asn Glu Ser Phe Lys Thr Ala Phe Gly Leu Gly Ala Ala
    290                 295                 300
tcg gag aat gcc aag ttg gag ttg gag aag cgt gga ctt cag tac cca        960
Ser Glu Asn Ala Lys Leu Glu Leu Glu Lys Arg Gly Leu Gln Tyr Pro
305                 310                 315                 320
ctt ttg gag aag ctt gga atc acc ctt cac tac act tgg atg ttc gtc       1008
Leu Leu Glu Lys Leu Gly Ile Thr Leu His Tyr Thr Trp Met Phe Val
                325                 330                 335
ctc tct tcc gga ttt gga agg tgg tct ctt cca tat tcc atc atg tat       1056
Leu Ser Ser Gly Phe Gly Arg Trp Ser Leu Pro Tyr Ser Ile Met Tyr
            340                 345                 350
ttc ttc act gcc aca tgc tcc tcg gga ctt ttc ctc gca ttg gtc ttt       1104
Phe Phe Thr Ala Thr Cys Ser Ser Gly Leu Phe Leu Ala Leu Val Phe
        355                 360                 365
gga ttg gga cac aac ggt atg tca gtg tac gat gcc acc acc cga cct       1152
Gly Leu Gly His Asn Gly Met Ser Val Tyr Asp Ala Thr Thr Arg Pro
    370                 375                 380
gac ttc tgg caa ctc caa gtc acc act aca cgt aac atc att ggt gga       1200
Asp Phe Trp Gln Leu Gln Val Thr Thr Thr Arg Asn Ile Ile Gly Gly
385                 390                 395                 400
cac ggc att ccc caa ttc ttt gtg gat tgg ttc tgc ggt gga ttg caa       1248
His Gly Ile Pro Gln Phe Phe Val Asp Trp Phe Cys Gly Gly Leu Gln
                405                 410                 415
tac caa gtg gat cac cac ctc ttc ccc atg atg cct aga aac aat atc       1296
Tyr Gln Val Asp His His Leu Phe Pro Met Met Pro Arg Asn Asn Ile
            420                 425                 430
gcg aag tgc cac aag ctt gtg gag tca ttc tgt aag gag tgg ggt gtg       1344
Ala Lys Cys His Lys Leu Val Glu Ser Phe Cys Lys Glu Trp Gly Val
        435                 440                 445
aag tac cat gag gct gat atg tgg gat ggt acc gtg gaa gtg ttg caa       1392
Lys Tyr His Glu Ala Asp Met Trp Asp Gly Thr Val Glu Val Leu Gln
    450                 455                 460
cat ctc tcc aag gtg tcg gat gat ttc ctt gtg gag atg gtg aag gat       1440
His Leu Ser Lys Val Ser Asp Asp Phe Leu Val Glu Met Val Lys Asp
465                 470                 475                 480
ttc cct gcc atg taa                                                   1455
Phe Pro Ala Met
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<210> 12
<211> 484
<212> PRT
<213> Thalassiosira pseudonana

<400> 12

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Met Gly Lys Gly Gly Asp Ala Ala Ala Ala Thr Lys Arg Ser Gly Ala
1               5                  10                  15
Leu Lys Leu Ala Glu Lys Pro Gln Lys Tyr Thr Trp Gln Glu Val Lys
            20                  25                  30
Lys His Ile Thr Pro Asp Asp Ala Trp Val Val His Gln Asn Lys Val
        35                  40                  45
Tyr Asp Val Ser Asn Trp Tyr Asp His Pro Gly Gly Ala Val Val Phe
    50                  55                  60
Thr His Ala Gly Asp Asp Met Thr Asp Ile Phe Ala Ala Phe His Ala
65                  70                  75                  80
Gln Gly Ser Gln Ala Met Met Lys Lys Phe Tyr Ile Gly Asp Leu Ile
                85                  90                  95
Pro Glu Ser Val Glu His Lys Asp Gln Arg Gln Leu Asp Phe Glu Lys
            100                 105                 110
Gly Tyr Arg Asp Leu Arg Ala Lys Leu Val Met Met Gly Met Phe Lys
        115                 120                 125
Ser Ser Lys Met Tyr Tyr Ala Tyr Lys Cys Ser Phe Asn Met Cys Met
    130                 135                 140
Trp Leu Val Ala Val Ala Met Val Tyr Tyr Ser Asp Ser Leu Ala Met
145                 150                 155                 160
His Ile Gly Ser Ala Leu Leu Leu Gly Leu Phe Trp Gln Gln Cys Gly
                165                 170                 175
Trp Leu Ala His Asp Phe Leu His His Gln Val Phe Lys Gln Arg Lys
            180                 185                 190
Tyr Gly Asp Leu Val Gly Ile Phe Trp Gly Asp Leu Met Gln Gly Phe
        195                 200                 205
Ser Met Gln Trp Trp Lys Asn Lys His Asn Gly His His Ala Val Pro
    210                 215                 220
Asn Leu His Asn Ser Ser Leu Asp Ser Gln Asp Gly Asp Pro Asp Ile
225                 230                 235                 240
Asp Thr Met Pro Leu Leu Ala Trp Ser Leu Lys Gln Ala Gln Ser Phe
                245                 250                 255
Arg Glu Ile Asn Lys Gly Lys Asp Ser Thr Phe Val Lys Tyr Ala Ile
            260                 265                 270
Lys Phe Gln Ala Phe Thr Tyr Phe Pro Ile Leu Leu Leu Ala Arg Ile
        275                 280                 285
Ser Trp Leu Asn Glu Ser Phe Lys Thr Ala Phe Gly Leu Gly Ala Ala
    290                 295                 300
Ser Glu Asn Ala Lys Leu Glu Leu Glu Lys Arg Gly Leu Gln Tyr Pro
305                 310                 315                 320
Leu Leu Glu Lys Leu Gly Ile Thr Leu His Tyr Thr Trp Met Phe Val
                325                 330                 335
Leu Ser Ser Gly Phe Gly Arg Trp Ser Leu Pro Tyr Ser Ile Met Tyr
            340                 345                 350
Phe Phe Thr Ala Thr Cys Ser Ser Gly Leu Phe Leu Ala Leu Val Phe
        355                 360                 365
Gly Leu Gly His Asn Gly Met Ser Val Tyr Asp Ala Thr Thr Arg Pro
    370                 375                 380
Asp Phe Trp Gln Leu Gln Val Thr Thr Thr Arg Asn Ile Ile Gly Gly
385                 390                 395                 400
His Gly Ile Pro Gln Phe Phe Val Asp Trp Phe Cys Gly Gly Leu Gln
                405                 410                 415
Tyr Gln Val Asp His His Leu Phe Pro Met Met Pro Arg Asn Asn Ile
            420                 425                 430
Ala Lys Cys His Lys Leu Val Glu Ser Phe Cys Lys Glu Trp Gly Val
        435                 440                 445
Lys Tyr His Glu Ala Asp Met Trp Asp Gly Thr Val Glu Val Leu Gln
    450                 455                 460
His Leu Ser Lys Val Ser Asp Asp Phe Leu Val Glu Met Val Lys Asp
465                 470                 475                 480
Phe Pro Ala Met
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<210> 13
<211> 1446
<212> DNA
<213> Marchantia polymorpha

<220>
<221> CDS
<222> (1)..(1446)

<400> 13

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atg gcc tcg tcc acc acc acc gcc gtg aag caa tct tcg ggt ggg ctg        48
Met Ala Ser Ser Thr Thr Thr Ala Val Lys Gln Ser Ser Gly Gly Leu
1               5                  10                  15
tgg tcg aaa tgg ggc acc ggc agc aac ttg agc ttc gtg tcg cgc aag        96
Trp Ser Lys Trp Gly Thr Gly Ser Asn Leu Ser Phe Val Ser Arg Lys
            20                  25                  30
gag cag cag cag cag cag cag cag agc tct ccc gag gcg tcg act ccc       144
Glu Gln Gln Gln Gln Gln Gln Gln Ser Ser Pro Glu Ala Ser Thr Pro
        35                  40                  45
gcg gcg cag cag gag aaa tcc atc agt aga gaa tcc atc ccc gag ggc       192
Ala Ala Gln Gln Glu Lys Ser Ile Ser Arg Glu Ser Ile Pro Glu Gly
    50                  55                  60
ttc ttg acc gtg gag gag gtg tcg aag cac gac aat ccg agc gac tgc       240
Phe Leu Thr Val Glu Glu Val Ser Lys His Asp Asn Pro Ser Asp Cys
65                  70                  75                  80
tgg atc gtc atc aac gac aag gtg tac gac gtg agc gca ttc ggg aag       288
Trp Ile Val Ile Asn Asp Lys Val Tyr Asp Val Ser Ala Phe Gly Lys
                85                  90                  95
acg cat ccg ggc ggc cct gtg atc ttc acg cag gcc ggc cgc gac gcc       336
Thr His Pro Gly Gly Pro Val Ile Phe Thr Gln Ala Gly Arg Asp Ala
            100                 105                 110
acg gat tct ttc aag gtt ttc cac tcc gcc aag gcg tgg cag ttt ctc       384
Thr Asp Ser Phe Lys Val Phe His Ser Ala Lys Ala Trp Gln Phe Leu
        115                 120                 125
cag gac ctg tac atc gga gat ctg tac aat gcc gag cca gtg tcg gag       432
Gln Asp Leu Tyr Ile Gly Asp Leu Tyr Asn Ala Glu Pro Val Ser Glu
    130                 135                 140
ctg gtg aag gat tac cga gac ctg agg acg gcg ttc atg cgt tct cag       480
Leu Val Lys Asp Tyr Arg Asp Leu Arg Thr Ala Phe Met Arg Ser Gln
145                 150                 155                 160
cta ttc aag agc agt aaa atg tac tac gtg acc aag tgc gtc aca aat       528
Leu Phe Lys Ser Ser Lys Met Tyr Tyr Val Thr Lys Cys Val Thr Asn
                165                 170                 175
ttt gca att ctt gcc gcc agt ctc gca gtc atc gcg tgg agc cag acg       576
Phe Ala Ile Leu Ala Ala Ser Leu Ala Val Ile Ala Trp Ser Gln Thr
            180                 185                 190
tat ctg gcg gtt ttg tgc tcc agt ttc ctg ttg gct ctc ttc tgg cag       624
Tyr Leu Ala Val Leu Cys Ser Ser Phe Leu Leu Ala Leu Phe Trp Gln
        195                 200                 205
caa tgt gga tgg tta tcg cac gat ttt ctc cac cac cag gtg acc gag       672
Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val Thr Glu
    210                 215                 220
aac cga tcg ctc aac acg tac ttc ggc ggc ctg ttc tgg ggt aac ttc       720
Asn Arg Ser Leu Asn Thr Tyr Phe Gly Gly Leu Phe Trp Gly Asn Phe
225                 230                 235                 240
gcc cag ggc tac agc gtg gga tgg tgg aag acc aag cac aat gtg cac       768
Ala Gln Gly Tyr Ser Val Gly Trp Trp Lys Thr Lys His Asn Val His
                245                 250                 255
cac gcg gcc acg aac gaa tgc gac gac aag tat cag ccc atc gat ccc       816
His Ala Ala Thr Asn Glu Cys Asp Asp Lys Tyr Gln Pro Ile Asp Pro
            260                 265                 270
gac atc gac acc gtg ccc ctg ctc gcc tgg agc aag gaa atc ttg gcc       864
Asp Ile Asp Thr Val Pro Leu Leu Ala Trp Ser Lys Glu Ile Leu Ala
        275                 280                 285
acc gtc gac gac caa ttc ttc cga tcg atc atc agc gtg cag cac ctt       912
Thr Val Asp Asp Gln Phe Phe Arg Ser Ile Ile Ser Val Gln His Leu
    290                 295                 300
ctg ttc ttc ccg ctc ctc ttc ttg gca aga ttc agc tgg ctg cat tcg       960
Leu Phe Phe Pro Leu Leu Phe Leu Ala Arg Phe Ser Trp Leu His Ser
305                 310                 315                 320
agt tgg gcc cac gcc agc aac ttc gag atg cct cgg tac atg aga tgg      1008
Ser Trp Ala His Ala Ser Asn Phe Glu Met Pro Arg Tyr Met Arg Trp
                325                 330                 335
```

```
gcg gag aag gcc tcg ctc ctc ggg cac tac ggc gcc tca atc ggc gcc        1056
Ala Glu Lys Ala Ser Leu Leu Gly His Tyr Gly Ala Ser Ile Gly Ala
                340                 345                 350
gcc ttc tac att ttg ccc atc ccc cag gcc atc tgc tgg ctc ttc ttg        1104
Ala Phe Tyr Ile Leu Pro Ile Pro Gln Ala Ile Cys Trp Leu Phe Leu
            355                 360                 365
tcg caa ctg ttt tgc ggc gct ctg ctc agc att gtc ttc gtg atc agc        1152
Ser Gln Leu Phe Cys Gly Ala Leu Leu Ser Ile Val Phe Val Ile Ser
        370                 375                 380
cac aat ggc atg gat gtg tac aac gac ccc cgg gac ttc gtg acg gcc        1200
His Asn Gly Met Asp Val Tyr Asn Asp Pro Arg Asp Phe Val Thr Ala
385                 390                 395                 400
caa gtc acc tcg acc aga aac atc gaa ggc aac ttc ttc aac gac tgg        1248
Gln Val Thr Ser Thr Arg Asn Ile Glu Gly Asn Phe Phe Asn Asp Trp
                405                 410                 415
ttc acc gga ggc ctg aac agg cag att gag cac cat ctg ttt ccg tct        1296
Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro Ser
            420                 425                 430
ctt ccg agg cac aac ctc gcc aag gtc gcg cca cac gtc aag gcg ctc        1344
Leu Pro Arg His Asn Leu Ala Lys Val Ala Pro His Val Lys Ala Leu
        435                 440                 445
tgc gcc aag cac ggt ttg cat tac gaa gaa ttg agt ctg ggc acg gga        1392
Cys Ala Lys His Gly Leu His Tyr Glu Glu Leu Ser Leu Gly Thr Gly
    450                 455                 460
gtc tgt cgt gtc ttc aat cgg cta gta gag gta gca tac gct gcg aaa        1440
Val Cys Arg Val Phe Asn Arg Leu Val Glu Val Ala Tyr Ala Ala Lys
465                 470                 475                 480
gta tag                                                             1446
Val
```

<210> 14
<211> 481
<212> PRT
<213> Marchantia polymorpha

<400> 14

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Met Ala Ser Ser Thr Thr Thr Ala Val Lys Gln Ser Ser Gly Gly Leu
1               5                  10                  15
Trp Ser Lys Trp Gly Thr Gly Ser Asn Leu Ser Phe Val Ser Arg Lys
            20                  25                  30
Glu Gln Gln Gln Gln Gln Gln Gln Ser Ser Pro Glu Ala Ser Thr Pro
        35                  40                  45
Ala Ala Gln Gln Glu Lys Ser Ile Ser Arg Glu Ser Ile Pro Glu Gly
    50                  55                  60
Phe Leu Thr Val Glu Glu Val Ser Lys His Asp Asn Pro Ser Asp Cys
65                  70                  75                  80
Trp Ile Val Ile Asn Asp Lys Val Tyr Asp Val Ser Ala Phe Gly Lys
                85                  90                  95
Thr His Pro Gly Gly Pro Val Ile Phe Thr Gln Ala Gly Arg Asp Ala
            100                 105                 110
Thr Asp Ser Phe Lys Val Phe His Ser Ala Lys Ala Trp Gln Phe Leu
        115                 120                 125
Gln Asp Leu Tyr Ile Gly Asp Leu Tyr Asn Ala Glu Pro Val Ser Glu
    130                 135                 140
Leu Val Lys Asp Tyr Arg Asp Leu Arg Thr Ala Phe Met Arg Ser Gln
145                 150                 155                 160
Leu Phe Lys Ser Ser Lys Met Tyr Tyr Val Thr Lys Cys Val Thr Asn
                165                 170                 175
Phe Ala Ile Leu Ala Ala Ser Leu Ala Val Ile Ala Trp Ser Gln Thr
            180                 185                 190
Tyr Leu Ala Val Leu Cys Ser Ser Phe Leu Leu Ala Leu Phe Trp Gln
        195                 200                 205
Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val Thr Glu
    210                 215                 220
Asn Arg Ser Leu Asn Thr Tyr Phe Gly Gly Leu Phe Trp Gly Asn Phe
225                 230                 235                 240
Ala Gln Gly Tyr Ser Val Gly Trp Trp Lys Thr Lys His Asn Val His
```

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                    245                 250                 255
His Ala Ala Thr Asn Glu Cys Asp Asp Lys Tyr Gln Pro Ile Asp Pro
                260                 265                 270
Asp Ile Asp Thr Val Pro Leu Leu Ala Trp Ser Lys Glu Ile Leu Ala
            275                 280                 285
Thr Val Asp Asp Gln Phe Phe Arg Ser Ile Ile Ser Val Gln His Leu
        290                 295                 300
Leu Phe Phe Pro Leu Leu Phe Leu Ala Arg Phe Ser Trp Leu His Ser
305                 310                 315                 320
Ser Trp Ala His Ala Ser Asn Phe Glu Met Pro Arg Tyr Met Arg Trp
                325                 330                 335
Ala Glu Lys Ala Ser Leu Leu Gly His Tyr Gly Ala Ser Ile Gly Ala
            340                 345                 350
Ala Phe Tyr Ile Leu Pro Ile Pro Gln Ala Ile Cys Trp Leu Phe Leu
        355                 360                 365
Ser Gln Leu Phe Cys Gly Ala Leu Leu Ser Ile Val Phe Val Ile Ser
    370                 375                 380
His Asn Gly Met Asp Val Tyr Asn Asp Pro Arg Asp Phe Val Thr Ala
385                 390                 395                 400
Gln Val Thr Ser Thr Arg Asn Ile Glu Gly Asn Phe Phe Asn Asp Trp
                405                 410                 415
Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro Ser
            420                 425                 430
Leu Pro Arg His Asn Leu Ala Lys Val Ala Pro His Val Lys Ala Leu
        435                 440                 445
Cys Ala Lys His Gly Leu His Tyr Glu Glu Leu Ser Leu Gly Thr Gly
    450                 455                 460
Val Cys Arg Val Phe Asn Arg Leu Val Glu Val Ala Tyr Ala Ala Lys
465                 470                 475                 480
Val
```

<210> 15
<211> 1377
<212> DNA
<213> Rhizopus oryzae

<220>
<221> CDS
<222> (1)..(1377)

<400> 15

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atg agt aca tca gat cgt caa tca gtt ttc aca tta aaa gag ttg gaa        48
Met Ser Thr Ser Asp Arg Gln Ser Val Phe Thr Leu Lys Glu Leu Glu
1               5                  10                  15
ctg att aat caa aaa cat cga gat gga gat aaa agt gcg atg aag ttc        96
Leu Ile Asn Gln Lys His Arg Asp Gly Asp Lys Ser Ala Met Lys Phe
            20                  25                  30
att atc att gat cgt aag gtg tac gat gtg act gaa ttc ttg gaa gat       144
Ile Ile Ile Asp Arg Lys Val Tyr Asp Val Thr Glu Phe Leu Glu Asp
        35                  40                  45
cat cct ggt ggt gca caa gta tta ctg aca cac gtt gga aaa gat gca       192
His Pro Gly Gly Ala Gln Val Leu Leu Thr His Val Gly Lys Asp Ala
    50                  55                  60
tct gat gta ttt cat gcc atg cat ccc gag tca gca tat gaa atc ttg       240
Ser Asp Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Ile Leu
65                  70                  75                  80
aac aat tat ttt gta gga gat gta aaa gat gca cat gta aag gag acc       288
Asn Asn Tyr Phe Val Gly Asp Val Lys Asp Ala His Val Lys Glu Thr
                85                  90                  95
cct tct gct caa ttt gct tca gaa atg cgt caa ctt cgg gat caa ttg       336
Pro Ser Ala Gln Phe Ala Ser Glu Met Arg Gln Leu Arg Asp Gln Leu
            100                 105                 110
aaa aaa gaa ggt tat ttt cat tct agt aaa gct tat tat gtt tac aag       384
Lys Lys Glu Gly Tyr Phe His Ser Ser Lys Ala Tyr Tyr Val Tyr Lys
        115                 120                 125
gtc ctc tct act ctt gct ctt tgt gca gct ggt ctt act ctt ttg tat       432
Val Leu Ser Thr Leu Ala Leu Cys Ala Ala Gly Leu Thr Leu Leu Tyr
```

```
    130                 135                 140
gct tat gga cat aca tct act tta gct gtt gtt gca tct gct att att        480
Ala Tyr Gly His Thr Ser Thr Leu Ala Val Val Ala Ser Ala Ile Ile
145                 150                 155                 160
gtt ggt atc ttt tgg caa caa tgt ggt tgg ttg gct cat gat ttt gga        528
Val Gly Ile Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Gly
                165                 170                 175
cat cat caa tgc ttt gaa gat cgc tct tgg aat gat gtt ctt gtt gtt        576
His His Gln Cys Phe Glu Asp Arg Ser Trp Asn Asp Val Leu Val Val
            180                 185                 190
ttc ctt gga aac ttt tgt caa ggt ttt tca ttg tca tgg tgg aag aat        624
Phe Leu Gly Asn Phe Cys Gln Gly Phe Ser Leu Ser Trp Trp Lys Asn
        195                 200                 205
aaa cat aac act cac cac gct agt acg aat gtt cat gga cat gat ccc        672
Lys His Asn Thr His His Ala Ser Thr Asn Val His Gly His Asp Pro
    210                 215                 220
gat att gac act gct cct gtc tta tta tgg gat gaa tat gca tct gca        720
Asp Ile Asp Thr Ala Pro Val Leu Leu Trp Asp Glu Tyr Ala Ser Ala
225                 230                 235                 240
gct tat tat gcc tca ctg gat gaa gaa ccc aca atg att tct cga ttc        768
Ala Tyr Tyr Ala Ser Leu Asp Glu Glu Pro Thr Met Ile Ser Arg Phe
                245                 250                 255
ctt gct gaa agt gtc ttg cct cat caa act cgt tat tac ttc ttt gtt        816
Leu Ala Glu Ser Val Leu Pro His Gln Thr Arg Tyr Tyr Phe Phe Val
            260                 265                 270
ctc ggc ttt gct cgt tta tca tgg gcc atc caa tcc tta ctc tac tca        864
Leu Gly Phe Ala Arg Leu Ser Trp Ala Ile Gln Ser Leu Leu Tyr Ser
        275                 280                 285
ttc aaa caa ggt gct att aac aag tct cat caa ctc aac ctc ttt gaa        912
Phe Lys Gln Gly Ala Ile Asn Lys Ser His Gln Leu Asn Leu Phe Glu
    290                 295                 300
cgc ttt tgt ctt gtt agt cac tgg act tta ttc act tac tgt aca ctt        960
Arg Phe Cys Leu Val Ser His Trp Thr Leu Phe Thr Tyr Cys Thr Leu
305                 310                 315                 320
gcc tgg tgt agc aac gtc tat cac atg att ttg ttc ttt ttg gtt agt       1008
Ala Trp Cys Ser Asn Val Tyr His Met Ile Leu Phe Phe Leu Val Ser
                325                 330                 335
caa gca act acc ggt tac aca ttg gcc ctt gta ttt gct ttg aat cac       1056
Gln Ala Thr Thr Gly Tyr Thr Leu Ala Leu Val Phe Ala Leu Asn His
            340                 345                 350
aat ggt atg cct gtg att act gaa gaa aag gcc gag tca atg gaa ttt       1104
Asn Gly Met Pro Val Ile Thr Glu Glu Lys Ala Glu Ser Met Glu Phe
        355                 360                 365
ttc gaa att caa gtg att aca ggt cgt gat gta aca ctc tct cct tta       1152
Phe Glu Ile Gln Val Ile Thr Gly Arg Asp Val Thr Leu Ser Pro Leu
    370                 375                 380
ggt gat tgg ttc atg ggt gga ttg aac tat caa atc gag cat cat gtt       1200
Gly Asp Trp Phe Met Gly Gly Leu Asn Tyr Gln Ile Glu His His Val
385                 390                 395                 400
ttc cct aat atg cct cgt cat aat tta cct aaa gta aag cca atg gtc       1248
Phe Pro Asn Met Pro Arg His Asn Leu Pro Lys Val Lys Pro Met Val
                405                 410                 415
aag tca ctt tgt aag aaa tat gat att aat tat cac gat act gga ttc       1296
Lys Ser Leu Cys Lys Lys Tyr Asp Ile Asn Tyr His Asp Thr Gly Phe
            420                 425                 430
ttg aaa gga aca ttg gaa gta cta aag aca tta gat att act tcc aag       1344
Leu Lys Gly Thr Leu Glu Val Leu Lys Thr Leu Asp Ile Thr Ser Lys
        435                 440                 445
ttg tct ttg caa ttg agc aaa aag tca ttt taa                          1377
Leu Ser Leu Gln Leu Ser Lys Lys Ser Phe
    450                 455
```

<210> 16
<211> 458
<212> PRT
<213> Rhizopus oryzae

<400> 16

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Met Ser Thr Ser Asp Arg Gln Ser Val Phe Thr Leu Lys Glu Leu Glu
1               5                  10                  15
Leu Ile Asn Gln Lys His Arg Asp Gly Asp Lys Ser Ala Met Lys Phe
            20                  25                  30
Ile Ile Ile Asp Arg Lys Val Tyr Asp Val Thr Glu Phe Leu Glu Asp
        35                  40                  45
His Pro Gly Gly Ala Gln Val Leu Leu Thr His Val Gly Lys Asp Ala
    50                  55                  60
Ser Asp Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Ile Leu
65                  70                  75                  80
Asn Asn Tyr Phe Val Gly Asp Val Lys Asp Ala His Val Lys Glu Thr
                85                  90                  95
Pro Ser Ala Gln Phe Ala Ser Glu Met Arg Gln Leu Arg Asp Gln Leu
            100                 105                 110
Lys Lys Glu Gly Tyr Phe His Ser Ser Lys Ala Tyr Tyr Val Tyr Lys
        115                 120                 125
Val Leu Ser Thr Leu Ala Leu Cys Ala Ala Gly Leu Thr Leu Leu Tyr
    130                 135                 140
Ala Tyr Gly His Thr Ser Thr Leu Ala Val Val Ala Ser Ala Ile Ile
145                 150                 155                 160
Val Gly Ile Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Gly
                165                 170                 175
His His Gln Cys Phe Glu Asp Arg Ser Trp Asn Asp Val Leu Val Val
            180                 185                 190
Phe Leu Gly Asn Phe Cys Gln Gly Phe Ser Leu Ser Trp Trp Lys Asn
        195                 200                 205
Lys His Asn Thr His His Ala Ser Thr Asn Val His Gly His Asp Pro
    210                 215                 220
Asp Ile Asp Thr Ala Pro Val Leu Leu Trp Asp Glu Tyr Ala Ser Ala
225                 230                 235                 240
Ala Tyr Tyr Ala Ser Leu Asp Glu Glu Pro Thr Met Ile Ser Arg Phe
                245                 250                 255
Leu Ala Glu Ser Val Leu Pro His Gln Thr Arg Tyr Tyr Phe Phe Val
            260                 265                 270
Leu Gly Phe Ala Arg Leu Ser Trp Ala Ile Gln Ser Leu Leu Tyr Ser
        275                 280                 285
Phe Lys Gln Gly Ala Ile Asn Lys Ser His Gln Leu Asn Leu Phe Glu
    290                 295                 300
Arg Phe Cys Leu Val Ser His Trp Thr Leu Phe Thr Tyr Cys Thr Leu
305                 310                 315                 320
Ala Trp Cys Ser Asn Val Tyr His Met Ile Leu Phe Phe Leu Val Ser
                325                 330                 335
Gln Ala Thr Thr Gly Tyr Thr Leu Ala Leu Val Phe Ala Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Thr Glu Glu Lys Ala Glu Ser Met Glu Phe
        355                 360                 365
Phe Glu Ile Gln Val Ile Thr Gly Arg Asp Val Thr Leu Ser Pro Leu
    370                 375                 380
Gly Asp Trp Phe Met Gly Gly Leu Asn Tyr Gln Ile Glu His His Val
385                 390                 395                 400
Phe Pro Asn Met Pro Arg His Asn Leu Pro Lys Val Lys Pro Met Val
                405                 410                 415
Lys Ser Leu Cys Lys Lys Tyr Asp Ile Asn Tyr His Asp Thr Gly Phe
            420                 425                 430
Leu Lys Gly Thr Leu Glu Val Leu Lys Thr Leu Asp Ile Thr Ser Lys
        435                 440                 445
Leu Ser Leu Gln Leu Ser Lys Lys Ser Phe
    450                 455
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<210> 17
<211> 1404
<212> DNA
<213> Mucor rouxii

<220>
<221> CDS
<222> (1)..(1404)

<400> 17

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atg agc agc gac gta gga gca aca gta ccc cat ttt tat act cgt gct        48
Met Ser Ser Asp Val Gly Ala Thr Val Pro His Phe Tyr Thr Arg Ala
1               5                  10                  15
gaa ttg gct gat atc cat caa gat gtt ctg gac aag aaa cct gaa gct        96
Glu Leu Ala Asp Ile His Gln Asp Val Leu Asp Lys Lys Pro Glu Ala
            20                  25                  30
aga aaa cta att gtc gtc gag aat aag gta tac gat atc aca gat ttc       144
Arg Lys Leu Ile Val Val Glu Asn Lys Val Tyr Asp Ile Thr Asp Phe
        35                  40                  45
gta ttt gat cac cct ggc ggt gag cga gta ctt ttg act caa gag ggc       192
Val Phe Asp His Pro Gly Gly Glu Arg Val Leu Leu Thr Gln Glu Gly
    50                  55                  60
aga gac gct aca gat gtc ttt cat gaa atg cat cct ccc tct gct tac       240
Arg Asp Ala Thr Asp Val Phe His Glu Met His Pro Pro Ser Ala Tyr
65                  70                  75                  80
gaa ttg ctg gcg aat tgc tat gtt ggc gat tgt gag ccc aag ctg cct       288
Glu Leu Leu Ala Asn Cys Tyr Val Gly Asp Cys Glu Pro Lys Leu Pro
                85                  90                  95
atc gat tca acc gat aag aag gca ttg aat tct gct gct ttt gct caa       336
Ile Asp Ser Thr Asp Lys Lys Ala Leu Asn Ser Ala Ala Phe Ala Gln
            100                 105                 110
gaa att cgt gat ctc cga gat aaa tta gaa aaa caa ggc tat ttc gac       384
Glu Ile Arg Asp Leu Arg Asp Lys Leu Glu Lys Gln Gly Tyr Phe Asp
        115                 120                 125
gct agt act ggt ttc tac atc tat aaa gtt tcc act acc cta ctt gtc       432
Ala Ser Thr Gly Phe Tyr Ile Tyr Lys Val Ser Thr Thr Leu Leu Val
    130                 135                 140
tgt att gta ggt tta gct atc ctc aaa gct tgg ggt aga gag tct act       480
Cys Ile Val Gly Leu Ala Ile Leu Lys Ala Trp Gly Arg Glu Ser Thr
145                 150                 155                 160
ttg gct gtg ttt att gct gct tct tta gtt ggt ctt ttt tgg cag cag       528
Leu Ala Val Phe Ile Ala Ala Ser Leu Val Gly Leu Phe Trp Gln Gln
                165                 170                 175
tgc ggt tgg ctt gcc cat gat tat gct cac tat caa gtc atc aaa gac       576
Cys Gly Trp Leu Ala His Asp Tyr Ala His Tyr Gln Val Ile Lys Asp
            180                 185                 190
ccc aat gtc aat aat ctc ttt tta gtt act ttt ggc aac ctg gtt caa       624
Pro Asn Val Asn Asn Leu Phe Leu Val Thr Phe Gly Asn Leu Val Gln
        195                 200                 205
ggt ttc tct ctt tca tgg tgg aaa aac aag cac aat acc cac cac gct       672
Gly Phe Ser Leu Ser Trp Trp Lys Asn Lys His Asn Thr His His Ala
    210                 215                 220
agc acc aat gtc tct ggc gaa gat ccc gat atc gat act gcc ccc att       720
Ser Thr Asn Val Ser Gly Glu Asp Pro Asp Ile Asp Thr Ala Pro Ile
225                 230                 235                 240
ttg cta tgg gac gag ttt gca gtc gct aat ttc tat gga tcc ttg aag       768
Leu Leu Trp Asp Glu Phe Ala Val Ala Asn Phe Tyr Gly Ser Leu Lys
                245                 250                 255
gac aat gcc agt gga ttt gac aga ttc att gct gaa cac att ttg ccc       816
Asp Asn Ala Ser Gly Phe Asp Arg Phe Ile Ala Glu His Ile Leu Pro
            260                 265                 270
tat caa act cgt tac tac ttc ttc att ctt ggt ttc gct cgt acc tct       864
Tyr Gln Thr Arg Tyr Tyr Phe Phe Ile Leu Gly Phe Ala Arg Thr Ser
        275                 280                 285
tgg gct atc caa tct att atc tat tcc ttc aag aac gaa aca ttg aat       912
Trp Ala Ile Gln Ser Ile Ile Tyr Ser Phe Lys Asn Glu Thr Leu Asn
    290                 295                 300
aaa tca aag ctc ttg tcc tgg tgt gag cgc atc ttt ttg att gtt cat       960
Lys Ser Lys Leu Leu Ser Trp Cys Glu Arg Ile Phe Leu Ile Val His
305                 310                 315                 320
tgg gtc ttt ttc act tac tgc act att gcc tgg atc agc tct atc aga      1008
Trp Val Phe Phe Thr Tyr Cys Thr Ile Ala Trp Ile Ser Ser Ile Arg
                325                 330                 335
aac atc gcc atg ttc ttt gtt gtt agc caa atc act act ggt tac ttg      1056
Asn Ile Ala Met Phe Phe Val Val Ser Gln Ile Thr Thr Gly Tyr Leu
            340                 345                 350
ctc gcc atc gtc ttt gct atg aac cat aat ggc atg cct gtt tac agc      1104
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Leu Ala Ile Val Phe Ala Met Asn His Asn Gly Met Pro Val Tyr Ser
            355                 360                 365
ccc gaa gaa gca aac cat acc gag ttt tat gaa ttg cag tgt atc act      1152
Pro Glu Glu Ala Asn His Thr Glu Phe Tyr Glu Leu Gln Cys Ile Thr
    370                 375                 380
ggt cgc gat gtc aac tgc act gta ttt ggc gat tgg ctc atg ggt gga      1200
Gly Arg Asp Val Asn Cys Thr Val Phe Gly Asp Trp Leu Met Gly Gly
385                 390                 395                 400
ttg aat tat caa att gag cac cat ctt ttc ccc gaa atg cct cga cat      1248
Leu Asn Tyr Gln Ile Glu His His Leu Phe Pro Glu Met Pro Arg His
                405                 410                 415
cat tta tcc aag gtg aaa tcc atg gtc aaa ccc att gct caa aag tat      1296
His Leu Ser Lys Val Lys Ser Met Val Lys Pro Ile Ala Gln Lys Tyr
            420                 425                 430
aat atc cct tac cat gat acc aca gtc att ggt ggt acc att gaa gtc      1344
Asn Ile Pro Tyr His Asp Thr Thr Val Ile Gly Gly Thr Ile Glu Val
            435                 440                 445
ttg caa acc ttg gat ttt gtt caa aag att tcg cag aaa ttc agc aaa      1392
Leu Gln Thr Leu Asp Phe Val Gln Lys Ile Ser Gln Lys Phe Ser Lys
    450                 455                 460
aaa atg ctc taa                                                     1404
Lys Met Leu
465

<210> 18
<211> 467
<212> PRT
<213> Mucor rouxii

<400> 18
Met Ser Ser Asp Val Gly Ala Thr Val Pro His Phe Tyr Thr Arg Ala
1               5                   10                  15
Glu Leu Ala Asp Ile His Gln Asp Val Leu Asp Lys Lys Pro Glu Ala
            20                  25                  30
Arg Lys Leu Ile Val Val Glu Asn Lys Val Tyr Asp Ile Thr Asp Phe
        35                  40                  45
Val Phe Asp His Pro Gly Gly Glu Arg Val Leu Leu Thr Gln Glu Gly
    50                  55                  60
Arg Asp Ala Thr Asp Val Phe His Glu Met His Pro Pro Ser Ala Tyr
65                  70                  75                  80
Glu Leu Leu Ala Asn Cys Tyr Val Gly Asp Cys Glu Pro Lys Leu Pro
                85                  90                  95
Ile Asp Ser Thr Asp Lys Lys Ala Leu Asn Ser Ala Ala Phe Ala Gln
            100                 105                 110
Glu Ile Arg Asp Leu Arg Asp Lys Leu Glu Lys Gln Gly Tyr Phe Asp
        115                 120                 125
Ala Ser Thr Gly Phe Tyr Ile Tyr Lys Val Ser Thr Thr Leu Leu Val
    130                 135                 140
Cys Ile Val Gly Leu Ala Ile Leu Lys Ala Trp Gly Arg Glu Ser Thr
145                 150                 155                 160
Leu Ala Val Phe Ile Ala Ala Ser Leu Val Gly Leu Phe Trp Gln Gln
                165                 170                 175
Cys Gly Trp Leu Ala His Asp Tyr Ala His Tyr Gln Val Ile Lys Asp
            180                 185                 190
Pro Asn Val Asn Asn Leu Phe Leu Val Thr Phe Gly Asn Leu Val Gln
        195                 200                 205
Gly Phe Ser Leu Ser Trp Trp Lys Asn Lys His Asn Thr His His Ala
    210                 215                 220
Ser Thr Asn Val Ser Gly Glu Asp Pro Asp Ile Asp Thr Ala Pro Ile
225                 230                 235                 240
Leu Leu Trp Asp Glu Phe Ala Val Ala Asn Phe Tyr Gly Ser Leu Lys
                245                 250                 255
Asp Asn Ala Ser Gly Phe Asp Arg Phe Ile Ala Glu His Ile Leu Pro
            260                 265                 270
Tyr Gln Thr Arg Tyr Tyr Phe Phe Ile Leu Gly Phe Ala Arg Thr Ser
        275                 280                 285
Trp Ala Ile Gln Ser Ile Ile Tyr Ser Phe Lys Asn Glu Thr Leu Asn
    290                 295                 300
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Lys Ser Lys Leu Leu Ser Trp Cys Glu Arg Ile Phe Leu Ile Val His
305                 310                 315                 320
Trp Val Phe Phe Thr Tyr Cys Thr Ile Ala Trp Ile Ser Ser Ile Arg
                325                 330                 335
Asn Ile Ala Met Phe Phe Val Val Ser Gln Ile Thr Thr Gly Tyr Leu
            340                 345                 350
Leu Ala Ile Val Phe Ala Met Asn His Asn Gly Met Pro Val Tyr Ser
        355                 360                 365
Pro Glu Glu Ala Asn His Thr Glu Phe Tyr Glu Leu Gln Cys Ile Thr
    370                 375                 380
Gly Arg Asp Val Asn Cys Thr Val Phe Gly Asp Trp Leu Met Gly Gly
385                 390                 395                 400
Leu Asn Tyr Gln Ile Glu His His Leu Phe Pro Glu Met Pro Arg His
                405                 410                 415
His Leu Ser Lys Val Lys Ser Met Val Lys Pro Ile Ala Gln Lys Tyr
            420                 425                 430
Asn Ile Pro Tyr His Asp Thr Thr Val Ile Gly Gly Thr Ile Glu Val
        435                 440                 445
Leu Gln Thr Leu Asp Phe Val Gln Lys Ile Ser Gln Lys Phe Ser Lys
    450                 455                 460
Lys Met Leu
465

<210> 19
<211> 1374
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(1374)

<400> 19
atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag att ttg        48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
aat gcc gag gct ctg aat gag ggc aag aag gat gcc gag gca ccc ttc        96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
ctg atg atc atc gac aac aag gtg tac gat gtc cgc gag ttc gtc cct       144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
gat cat ccc ggt gga agt gtg att ctc acg cac gtt ggc aag gac ggc       192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
act gac gtc ttt gac act ttt cac ccc gag gct gct tgg gag act ctt       240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tac gtt ggt gat att gac gag agc gac cgc gat atc aag       288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
                85                  90                  95
aat gat gac ttt gcg gcc gag gtc cgc aag ctg cgt acc ttg ttc cag       336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
tct ctt ggt tac tac gat tct tcc aag gca tac tac gcc ttc aag gtc       384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
tcg ttc aac ctc tgc atc tgg ggt ttg tcg acg gtc att gtg gcc aag       432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
    130                 135                 140
tgg ggc cag acc tcg acc ctc gcc aac gtg ctc tcg gct gcg ctt ttg       480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctg ttc tgg cag cag tgc gga tgg ttg gct cac gac ttt ttg cat       528
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc       576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
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                 180                 185                 190
ttg gga ggt gtc tgc cag ggc ttc tcg tcc tcg tgg tgg aag gac aag        624
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
            195                 200                 205
cac aac act cac cac gcc gcc ccc aac gtc cac ggc gaa gat ccc gac        672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
        210                 215                 220
att gac acc cac cct ctg ttg acc tgg agt gag cat gcg ttg gag atg        720
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
ttc tcg gat gtc cca gat gag gag ctg acc cgc atg tgg tcg cgt ttc        768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctg aac cag acc tgg ttt tac ttc ccc att ctc tcg ttt gcc        816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctc tcc tgg tgc ctc cag tcc att ctc ttt gtg ctg cct aac ggt        864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
            275                 280                 285
cag gcc cac aag ccc tcg ggc gcg cgt gtg ccc atc tcg ttg gtc gag        912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
        290                 295                 300
cag ctg tcg ctt gcg atg cac tgg acc tgg tac ctc gcc acc atg ttc        960
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ctg ttc atc aag gat ccc gtc aac atg ctg gtg tac ttt ttg gtg tcg       1008
Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
                325                 330                 335
cag ccg gtg tgc gga aac ttg ttg gcg atc gtg ttc tcg ctc aac cac       1056
Gln Pro Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggt atg cct gtg atc tcg aag gag gag gcg gtc gat atg gat ttc       1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
            355                 360                 365
ttc acg aag cag atc atc acg ggt cgt gat gtc cac ccg ggt cta ttt       1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
        370                 375                 380
gcc aac tgg ttc acg ggt gga ttg aac tat cag atc gag cac cac ttg       1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc       1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag acc ctg tgc aaa aag tac aat gtc cga tac cac acc acc ggt atg       1296
Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ctg aac gag gtc tcc aag       1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
            435                 440                 445
gct gcc tcc aag atg ggc aag gca cag taa                              1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
        450                 455

<210> 20
<211> 457
<212> PRT
<213> Mortierella alpina

<400> 20
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
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65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
    130                 135                 140
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Pro Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 21
<211> 1377
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(1377)

<400> 21
atg acc acc agc gac cca tct gtc aga gcg ttc aca cgc tca gaa gtg        48
Met Thr Thr Ser Asp Pro Ser Val Arg Ala Phe Thr Arg Ser Glu Val
1               5                  10                  15
ttg cac gcc gat gcc ttg aac gag ggc aaa aag aac gcc gag gca ccg        96
Leu His Ala Asp Ala Leu Asn Glu Gly Lys Lys Asn Ala Glu Ala Pro
            20                  25                  30
ttt ctc atg atc atc gac aac aag gtc tac gat gtg cgc gag ttt atc       144
Phe Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Ile
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                35                  40                  45
ccc gac cat cct ggt ggg agc gtc att ttg acc cac gta ggc aag gac        192
Pro Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp
    50                  55                  60
ggc acc gac gtt ttc gag acc ttc cat cct gag gct gct tgg gag acg        240
Gly Thr Asp Val Phe Glu Thr Phe His Pro Glu Ala Ala Trp Glu Thr
65                  70                  75                  80
ctc gcc aat ttt tat gtc ggt gac att gta gaa tcc gat cgc gcc atc        288
Leu Ala Asn Phe Tyr Val Gly Asp Ile Val Glu Ser Asp Arg Ala Ile
                85                  90                  95
gag aac gac gag ttt gca gct gag gtt cgt aag ctg cgg aca ttg ttt        336
Glu Asn Asp Glu Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe
            100                 105                 110
tat tct ttg ggc tac tac gac tca tcc aag gtt tac tac gcc ttc aag        384
Tyr Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Val Tyr Tyr Ala Phe Lys
        115                 120                 125
gtc tcg ttc aac ctc tgc atc tgg ggc ctg tct gca ttc att gtt gcc        432
Val Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Ala Phe Ile Val Ala
    130                 135                 140
aaa tgg ggc cag acc tcg acc ctc gca aac gtg ata tca gcc tca ctc        480
Lys Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Ile Ser Ala Ser Leu
145                 150                 155                 160
ctg ggt gtc ttt tgg caa cag tgc ggt tgg ctc gcc cat gat ttc ttg        528
Leu Gly Val Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu
                165                 170                 175
cac cat cag gtc ttt cac gat cga ttc tgg ggc gat ctg ttc ggt gca        576
His His Gln Val Phe His Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala
            180                 185                 190
ttt ctc ggc gga gtc tgt caa ggt ttc tcc tcg tcc tgg tgg aag gac        624
Phe Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp
        195                 200                 205
aaa cac aac acc cac cac gcg gcg ccc aat gtc cat gga gag gat ccc        672
Lys His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro
    210                 215                 220
gat atc gac aca cat ccg ctt ttg acg tgg agt gag cat gcg ctc gag        720
Asp Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu
225                 230                 235                 240
atg ttt tcg gat gtg ccc gat gag gag ctt acc caa atg tgg tcc cgg        768
Met Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Gln Met Trp Ser Arg
                245                 250                 255
ttt atg gtt ctg aac cag gcc tgg ttt tac ttt ccc att ctg tca ttt        816
Phe Met Val Leu Asn Gln Ala Trp Phe Tyr Phe Pro Ile Leu Ser Phe
            260                 265                 270
gcc cgc ctg tcc tgg tgc atc cag tcg att ctt ttt gtg cta ccg aac        864
Ala Arg Leu Ser Trp Cys Ile Gln Ser Ile Leu Phe Val Leu Pro Asn
        275                 280                 285
gga cag gca cac aaa cct gcg ggg gct cgg gtt ccc atc tcg ctg gtg        912
Gly Gln Ala His Lys Pro Ala Gly Ala Arg Val Pro Ile Ser Leu Val
    290                 295                 300
gag caa ttg tcg ttg gcg atg cac tgg acc tgg tac ctg gca acc atg        960
Glu Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met
305                 310                 315                 320
ttc ctg ttc atc aag gat ccc gtc aac atg atg gtg tat ttc ttg gtc       1008
Phe Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val
                325                 330                 335
tcg caa gct gtc tgc ggc aac ctg tta gcg att gtg ttc tcg ctg aac       1056
Ser Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn
            340                 345                 350
cat aac ggt atg cct gtg atc tcg cag gag gaa gcg gtc gag atg gat       1104
His Asn Gly Met Pro Val Ile Ser Gln Glu Glu Ala Val Glu Met Asp
        355                 360                 365
ttc ttc aca aag cag atc att acg ggt cgt gat gtc tac ccg ggt tgg       1152
Phe Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val Tyr Pro Gly Trp
    370                 375                 380
ttt gca gac tgg ttc acg ggt gga ttg aac tat cag att gaa cac cat       1200
Phe Ala Asp Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His
385                 390                 395                 400
ctg ttc ccg tcg atg cct cga cac cat ttc tca aag atc cag ccc gcg       1248
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Leu Phe Pro Ser Met Pro Arg His His Phe Ser Lys Ile Gln Pro Ala
                405                 410                 415
gtt gaa tcg ctg tgc aag aag tac ggg gtc cga tac cat acg acg ggg      1296
Val Glu Ser Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly
            420                 425                 430
atg att gct ggc acc gca gag gtc ttt tcg cga ctg aac gag gtg tcc      1344
Met Ile Ala Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser
        435                 440                 445
cag gct gca agc aag ctc ggc aag tct gct tga                          1377
Gln Ala Ala Ser Lys Leu Gly Lys Ser Ala
    450                 455

<210> 22
<211> 458
<212> PRT
<213> Mortierella alpina

<400> 22
Met Thr Thr Ser Asp Pro Ser Val Arg Ala Phe Thr Arg Ser Glu Val
1               5                  10                  15
Leu His Ala Asp Ala Leu Asn Glu Gly Lys Lys Asn Ala Glu Ala Pro
            20                  25                  30
Phe Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Ile
        35                  40                  45
Pro Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp
    50                  55                  60
Gly Thr Asp Val Phe Glu Thr Phe His Pro Glu Ala Ala Trp Glu Thr
65                  70                  75                  80
Leu Ala Asn Phe Tyr Val Gly Asp Ile Val Glu Ser Asp Arg Ala Ile
                85                  90                  95
Glu Asn Asp Glu Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe
            100                 105                 110
Tyr Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Val Tyr Tyr Ala Phe Lys
        115                 120                 125
Val Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Ala Phe Ile Val Ala
    130                 135                 140
Lys Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Ile Ser Ala Ser Leu
145                 150                 155                 160
Leu Gly Val Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu
                165                 170                 175
His His Gln Val Phe His Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala
            180                 185                 190
Phe Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp
        195                 200                 205
Lys His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro
    210                 215                 220
Asp Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu
225                 230                 235                 240
Met Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Gln Met Trp Ser Arg
                245                 250                 255
Phe Met Val Leu Asn Gln Ala Trp Phe Tyr Phe Pro Ile Leu Ser Phe
            260                 265                 270
Ala Arg Leu Ser Trp Cys Ile Gln Ser Ile Leu Phe Val Leu Pro Asn
        275                 280                 285
Gly Gln Ala His Lys Pro Ala Gly Ala Arg Val Pro Ile Ser Leu Val
    290                 295                 300
Glu Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met
305                 310                 315                 320
Phe Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val
                325                 330                 335
Ser Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn
            340                 345                 350
His Asn Gly Met Pro Val Ile Ser Gln Glu Glu Ala Val Glu Met Asp
        355                 360                 365
Phe Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val Tyr Pro Gly Trp
    370                 375                 380
Phe Ala Asp Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His
385                 390                 395                 400
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Leu Phe Pro Ser Met Pro Arg His His Phe Ser Lys Ile Gln Pro Ala
                405                 410                 415
Val Glu Ser Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly
            420                 425                 430
Met Ile Ala Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser
        435                 440                 445
Gln Ala Ala Ser Lys Leu Gly Lys Ser Ala
    450                 455

<210> 23
<211> 1434
<212> DNA
<213> Phaeodactylum tricornutum

<220>
<221> CDS
<222> (1)..(1434)

<400> 23
atg ggc aaa gga ggg gac gct cgg gcc tcg aag ggc tca acg gcg gct        48
Met Gly Lys Gly Gly Asp Ala Arg Ala Ser Lys Gly Ser Thr Ala Ala
1               5                   10                  15
cgc aag atc agt tgg cag gaa gtc aag acc cac gcg tct ccg gag gac        96
Arg Lys Ile Ser Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp
            20                  25                  30
gcc tgg atc att cac tcc aat aag gtc tac gac gtg tcc aac tgg cac       144
Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His
        35                  40                  45
gaa cat ccc gga ggc gcc gtc att ttc acg cac gcc ggt gac gac atg       192
Glu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
    50                  55                  60
acg gac att ttc gct gcc ttt cac gca ccc gga tcg cag tcg ctc atg       240
Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met
65                  70                  75                  80
aag aag ttc tac att ggc gaa ttg ctc ccg gaa acc acc ggc aag gag       288
Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu
                85                  90                  95
ccg cag caa atc gcc ttt gaa aag ggc tac cgc gat ctg cgc tcc aaa       336
Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys
            100                 105                 110
ctc atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac       384
Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
        115                 120                 125
aag tgc ctc agc aac atg gcc att tgg gcc gcc gcc tgt gct ctc gtc       432
Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
    130                 135                 140
ttt tac tcg gac cgc ttc tgg gta cac ctg gcc agc gcc gtc atg ctg       480
Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
145                 150                 155                 160
gga aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac       528
Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc acc aag cgc aag cac ggg gat ctc gga gga ctc ttt       576
His Gln Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
            180                 185                 190
tgg ggg aac ctc atg cag ggt tac tcc gta cag tgg tgg aaa aac aag       624
Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
        195                 200                 205
cac aac gga cac cac gcc gtc ccc aac ctc cac tgc tcc tcc gca gtc       672
His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
    210                 215                 220
gcg caa gat ggg gac ccg gac atc gat acc atg ccc ctt ctc gcc tgg       720
Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
225                 230                 235                 240
tcc gtc cag caa gcc cag tct tac cgg gaa ctc caa gcc gac gga aag       768
Ser Val Gln Gln Ala Gln Ser Tyr Arg Glu Leu Gln Ala Asp Gly Lys
                245                 250                 255
gat tcg ggt ttg gtc aag ttc atg atc cgt aac caa tcc tac ttt tac       816
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Asp Ser Gly Leu Val Lys Phe Met Ile Arg Asn Gln Ser Tyr Phe Tyr
                260                 265                 270
ttt ccc atc ttg ttg ctc gcc cgc ctg tcg tgg ttg aac gag tcc ttc        864
Phe Pro Ile Leu Leu Leu Ala Arg Leu Ser Trp Leu Asn Glu Ser Phe
        275                 280                 285
aag tgc gcc ttt ggg ctt gga gct gcg tcg gag aac gct gct ctc gaa        912
Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Glu Asn Ala Ala Leu Glu
    290                 295                 300
ctc aag gcc aag ggt ctt cag tac ccc ctt ttg gaa aag gct ggc atc        960
Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Glu Lys Ala Gly Ile
305                 310                 315                 320
ctg ctg cac tac gct tgg atg ctt aca gtt tcg tcc ggc ttt gga cgc       1008
Leu Leu His Tyr Ala Trp Met Leu Thr Val Ser Ser Gly Phe Gly Arg
                325                 330                 335
ttc tcg ttc gcg tac acc gca ttt tac ttt cta acc gcg acc gcg tcc       1056
Phe Ser Phe Ala Tyr Thr Ala Phe Tyr Phe Leu Thr Ala Thr Ala Ser
            340                 345                 350
tgt gga ttc ttg ctc gcc att gtc ttt ggc ctc ggc cac aac ggc atg       1104
Cys Gly Phe Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met
        355                 360                 365
gcc acc tac aat gcc gac gcc cgt ccg gac ttc tgg aag ctc caa gtc       1152
Ala Thr Tyr Asn Ala Asp Ala Arg Pro Asp Phe Trp Lys Leu Gln Val
    370                 375                 380
acc acg act cgc aac gtc acg ggc gga cac ggt ttc ccc caa gcc ttt       1200
Thr Thr Thr Arg Asn Val Thr Gly Gly His Gly Phe Pro Gln Ala Phe
385                 390                 395                 400
gtc gac tgg ttc tgt ggt ggc ctc cag tac caa gtc gac cac cac tta       1248
Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu
                405                 410                 415
ttc ccc agc ctg ccc cga cac aat ctg gcc aag aca cac gca ctg gtc       1296
Phe Pro Ser Leu Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val
            420                 425                 430
gaa tcg ttc tgc aag gag tgg ggt gtc cag tac cac gaa gcc gac ctt       1344
Glu Ser Phe Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu
        435                 440                 445
gtg gac ggg acc atg gaa gtc ttg cac cat ttg ggc agc gtg gcc ggc       1392
Val Asp Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly
    450                 455                 460
gaa ttc gtc gtg gat ttt gta cgc gat gga ccc gcc atg taa               1434
Glu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
465                 470                 475

<210> 24
<211> 477
<212> PRT
<213> Phaeodactylum tricornutum

<400> 24
Met Gly Lys Gly Gly Asp Ala Arg Ala Ser Lys Gly Ser Thr Ala Ala
1               5                   10                  15
Arg Lys Ile Ser Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp
            20                  25                  30
Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His
        35                  40                  45
Glu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
    50                  55                  60
Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met
65                  70                  75                  80
Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu
                85                  90                  95
Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys
            100                 105                 110
Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
        115                 120                 125
Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
    130                 135                 140
Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
145                 150                 155                 160
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Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
            180                 185                 190
Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
        195                 200                 205
His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
    210                 215                 220
Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
225                 230                 235                 240
Ser Val Gln Gln Ala Gln Ser Tyr Arg Glu Leu Gln Ala Asp Gly Lys
                245                 250                 255
Asp Ser Gly Leu Val Lys Phe Met Ile Arg Asn Gln Ser Tyr Phe Tyr
            260                 265                 270
Phe Pro Ile Leu Leu Leu Ala Arg Leu Ser Trp Leu Asn Glu Ser Phe
        275                 280                 285
Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Glu Asn Ala Ala Leu Glu
    290                 295                 300
Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Glu Lys Ala Gly Ile
305                 310                 315                 320
Leu Leu His Tyr Ala Trp Met Leu Thr Val Ser Ser Gly Phe Gly Arg
                325                 330                 335
Phe Ser Phe Ala Tyr Thr Ala Phe Tyr Phe Leu Thr Ala Thr Ala Ser
            340                 345                 350
Cys Gly Phe Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met
        355                 360                 365
Ala Thr Tyr Asn Ala Asp Ala Arg Pro Asp Phe Trp Lys Leu Gln Val
    370                 375                 380
Thr Thr Thr Arg Asn Val Thr Gly Gly His Gly Phe Pro Gln Ala Phe
385                 390                 395                 400
Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu
                405                 410                 415
Phe Pro Ser Leu Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val
            420                 425                 430
Glu Ser Phe Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu
        435                 440                 445
Val Asp Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly
    450                 455                 460
Glu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
465                 470                 475

<210> 25
<211> 1374
<212> DNA
<213> Mortierella isabellina

<220>
<221> CDS
<222> (1)..(1374)

<400> 25
atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag att ctg        48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
aat gcc gag gcc ctg aat gag ggc aag aag gat gcc gag gca cct ttc        96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
ttg atg atc atc gac aac aag gtg tat gat gtc cgc gag ttt gtc cct       144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
gat cat ccc ggt gga agt gtt att ctc acg cac gtt ggc aag gac ggc       192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
act gac gtc ttt gac act ttc cac ccc gag gct gct tgg gag aca ctt       240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tac gtt ggt gat att gat gag agc gac cgt gcc atc aag       288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
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                                85                  90                  95
aac gat gac ttt gcg gcc gag gtc cgc aag ctg cgt act ttg ttc cag          336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
                            100                 105                 110
tct ctg ggt tac tac gat tcc tcc aag gca tac tac gcc ttc aag gtc          384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
                        115                 120                 125
tca ttc aac ctc tgc atc tgg ggc ctg tcg acg ttc att gtt gcc aag          432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
tgg ggc cag acc tcg acc ctc gcc aac gtg ctc tcg gct gcg ctc ttg          480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctc ttc tgg cag cag tgt gga tgg ttg gcg cac gac ttt ttg cac          528
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc          576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
ttg gga ggt gtc tgc cag ggc ttc tcg tcc tca tgg tgg aag gac aag          624
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
cac aac act cac cac gcc gcc ccc aac gtc cac ggc gag gat ccc gac          672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
att gac act cac cct ctg ttg acg tgg agt gag cat gct ttg gag atg          720
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
ttc tcg gac gtc cct gac gag gag ctg acc cgc atg tgg tcg cgc ttc          768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctt aac cag acc tgg ttc tac ttt ccc att ctc tcg ttt gcc          816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctc tcc tgg tgc ctc cag tcc atc ctc ttt gtt ctg cct aac ggt          864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
cag gcc cac aag ccc tct gga gcc cgt gtg ccc att tcc ttg gtc gag          912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
cag ctg tct ctt gcc gtg cac tgg acc tgg tac ctc gcc acc atg ttc          960
Gln Leu Ser Leu Ala Val His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ttg ttc att aag gac ccc gtc aac atg atg gtg tac ttt ttg gtg tct         1008
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
cag gct gtt tgc ggt aac ctg ttg gcg att gtg ttc tcg ctc aac cac         1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggt atg cct gtg atc tcc aag gag gaa gcc gtc gac atg gat ttc         1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
ttc acc aag cag atc atc acg ggt cgt gat gtt cac cct ggt ctg ttc         1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
gcc aac tgg ttc acg ggt gga ttg aac tac cag att gag cac cac ttg         1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc         1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag acc ttg tgc aaa aag tat ggt gtc cga tac cac acc act ggc atg         1296
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ttg aac gag gtc tcc aag         1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
gcc gcc tcc aag atg ggt aag gcg cag taa                                 1374
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Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 26
<211> 457
<212> PRT
<213> Mortierella isabellina

<400> 26
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Val His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455
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<210> 27
<211> 1374
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(1374)

<400> 27

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atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag att ctg        48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
aat gcc gag gcc ctg aat gag ggc aag aag gat gcc gag gca cct ttc        96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
ttg atg atc atc gac aac aag gtg tat gat gtc cgc gag ttt gtc cct       144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
gat cat ccc ggt gga agt gtt att ctc acg cac gtt ggc aag gac ggc       192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
act gac gtc ttt gac act ttc cac ccc gag gct gct tgg gag aca ctt       240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tac gtt ggt gat att gat gag agc gac cgt gcc atc aag       288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
aac gat gac ttt gcg gcc gag gtc cgc aag ctg cgt act ttg ttc cag       336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
tct ctg ggt tac tac gat tcc tcc aag gca tac tac gcc ttc aag gtc       384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
tca ttc aac ctc tgc atc tgg ggc ctg tcg acg ttc att gtt gcc aag       432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
tgg ggc cag acc tcg acc ctc gcc aac gag ctc tcg gct gcg ctc ttg       480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Glu Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctc ttc tgg cag cag aga gga tgg ttg gcg cac gac ttt ttg cac       528
Gly Leu Phe Trp Gln Gln Arg Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg gga gat ctt ttc ggc gcc ttc       576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
ttg gga gga gac tgc cag ggc ttc tcg tcc tca tgg tgg aag gac aag       624
Leu Gly Gly Asp Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
cac aac act cac cac gcc gcc ccc aac gtc cac ggc gag gat ccc gac       672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
att gac act cac cct ctg ttg acg tgg agt gag cat gct ttg gag atg       720
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
ttc tcg gac gtc cct gac gag gag ctg acc cgc atg tgg tcg cgc ttc       768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctt aac cag acc tgg ttc tac ttt ccc att ctc tcg ttt gcc       816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctc tcc tgg tgc ctc cag tcc atc ctc ttt gtt ctg cct aac ggt       864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
cag gcc cac aag ccc tct gga gcc cgt gtg ccc att tcc ttg gtc gag       912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
cag ctg tct ctt gcc atg cac tgg acc tgg tac ctc gcc acc atg ttc       960
```

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Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ttg ttc att aag gac ccc gtc aac atg atg gtg tac ttt ttg gtg tct        1008
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
cag gct gtt tgc ggt aac ctg ttg gcg att gtg ttc tcg ctc aac cac        1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggt atg cct gtg atc tcc aag gag gaa gcc gtc gac atg gat ttc        1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
ttc acc aag cag atc atc acg ggt cgt gat gtt cac cct ggt ctg ttc        1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
gcc aac tgg ttc acg ggt gga ttg aac tac cag att gag cac cac ttg        1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc        1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag acc ttg tgc aaa aag tat ggt gtc cga tac cac acc act ggc atg        1296
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ttg aac gag gtc tcc aag        1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
gcc gcc tcc aag atg ggt aag gcg cag taa                                1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 28
<211> 457
<212> PRT
<213> Mortierella alpina

<400> 28
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                   10                  15
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
Trp Gly Gln Thr Ser Thr Leu Ala Asn Glu Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Arg Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Asp Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
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Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
                260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
            275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 29
<211> 1380
<212> DNA
<213> Pythium irregulare

<220>
<221> CDS
<222> (1)..(1380)

<400> 29
atg gtg gac ctc aag cct gga gtg aag cgc ctg gtg agc tgg aag gag        48
Met Val Asp Leu Lys Pro Gly Val Lys Arg Leu Val Ser Trp Lys Glu
1               5                   10                  15
atc cgc gag cac gcg acg ccc gcg acc gcg tgg atc gtg att cac cac        96
Ile Arg Glu His Ala Thr Pro Ala Thr Ala Trp Ile Val Ile His His
            20                  25                  30
aag gtc tac gac atc tcc aag tgg gac tcg cac ccg ggt ggc tcc gtg       144
Lys Val Tyr Asp Ile Ser Lys Trp Asp Ser His Pro Gly Gly Ser Val
        35                  40                  45
atg ctc acg cag gcc ggc gag gac gcc acg gac gcc ttc gcg gtc ttc       192
Met Leu Thr Gln Ala Gly Glu Asp Ala Thr Asp Ala Phe Ala Val Phe
    50                  55                  60
cac ccg tcc tcg gcg ctc aag ctg ctc gag cag ttc tac gtc ggc gac       240
His Pro Ser Ser Ala Leu Lys Leu Leu Glu Gln Phe Tyr Val Gly Asp
65                  70                  75                  80
gtg gac gaa acc tcc aag gcc gag atc gag ggg gag ccg gcg agc gac       288
Val Asp Glu Thr Ser Lys Ala Glu Ile Glu Gly Glu Pro Ala Ser Asp
                85                  90                  95
gag gag cgc gcg cgc cgc gag cgc atc aac gag ttc atc gcg tcc tac       336
Glu Glu Arg Ala Arg Arg Glu Arg Ile Asn Glu Phe Ile Ala Ser Tyr
            100                 105                 110
cgt cgt ctg cgc gtc aag gtc aag ggc atg ggg ctc tac gac gcc agc       384
Arg Arg Leu Arg Val Lys Val Lys Gly Met Gly Leu Tyr Asp Ala Ser
        115                 120                 125
gcg ctc tac tac gcg tgg aag ctc gtg agc acg ttc ggc atc gcg gtg       432
Ala Leu Tyr Tyr Ala Trp Lys Leu Val Ser Thr Phe Gly Ile Ala Val
    130                 135                 140
ctc tcg atg gcg atc tgc ttc ttc ttc aac agt ttc gcc atg tac atg       480
Leu Ser Met Ala Ile Cys Phe Phe Phe Asn Ser Phe Ala Met Tyr Met
145                 150                 155                 160
gtc gcc ggc gtg att atg ggg ctc ttc tac cag cag tcc gga tgg ctg       528
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Val Ala Gly Val Ile Met Gly Leu Phe Tyr Gln Gln Ser Gly Trp Leu
                165                 170                 175
gcg cac gac ttc ttg cac aac cag gtg tgc gag aac cgc acg ctc ggc        576
Ala His Asp Phe Leu His Asn Gln Val Cys Glu Asn Arg Thr Leu Gly
            180                 185                 190
aac ctt atc ggc tgc ctc gtg ggc aac gcc tgg cag ggc ttc agc gtg        624
Asn Leu Ile Gly Cys Leu Val Gly Asn Ala Trp Gln Gly Phe Ser Val
        195                 200                 205
cag tgg tgg aag aac aag cac aac ctg cac cac gcg gtg ccg aac ctg        672
Gln Trp Trp Lys Asn Lys His Asn Leu His His Ala Val Pro Asn Leu
    210                 215                 220
cac agc gcc aag gac gag ggc ttc atc ggc gac ccg gac atc gac acc        720
His Ser Ala Lys Asp Glu Gly Phe Ile Gly Asp Pro Asp Ile Asp Thr
225                 230                 235                 240
atg ccg ctg ctg gcg tgg tct aag gag atg gcg cgc aag gcg ttc gag        768
Met Pro Leu Leu Ala Trp Ser Lys Glu Met Ala Arg Lys Ala Phe Glu
                245                 250                 255
tcg gcg cac ggc ccg ttc ttc atc cgc aac cag gcg ttc cta tac ttc        816
Ser Ala His Gly Pro Phe Phe Ile Arg Asn Gln Ala Phe Leu Tyr Phe
            260                 265                 270
ccg ctg ctg ctg ctc gcg cgc ctg agc tgg ctc gcg cag tcg ttc ttc        864
Pro Leu Leu Leu Leu Ala Arg Leu Ser Trp Leu Ala Gln Ser Phe Phe
        275                 280                 285
tac gtg ttc acc gag ttc tcg ttc ggc atc ttc gac aag gtc gag ttc        912
Tyr Val Phe Thr Glu Phe Ser Phe Gly Ile Phe Asp Lys Val Glu Phe
    290                 295                 300
gac gga ccg gag aag gcg ggt ctg atc gtg cac tac atc tgg cag ctc        960
Asp Gly Pro Glu Lys Ala Gly Leu Ile Val His Tyr Ile Trp Gln Leu
305                 310                 315                 320
gcg atc ccg tac ttc tgc aac atg agc ctg ttt gag ggc gtg gca tac       1008
Ala Ile Pro Tyr Phe Cys Asn Met Ser Leu Phe Glu Gly Val Ala Tyr
                325                 330                 335
ttc ctc atg ggc cag gcg tcc tgc ggc ttg ctc ctg gcg ctg gtg ttc       1056
Phe Leu Met Gly Gln Ala Ser Cys Gly Leu Leu Leu Ala Leu Val Phe
            340                 345                 350
agt att ggc cac aac ggc atg tcg gtg tac gag cgc gaa acc aag ccg       1104
Ser Ile Gly His Asn Gly Met Ser Val Tyr Glu Arg Glu Thr Lys Pro
        355                 360                 365
gac ttc tgg cag ctg cag gtg acc acg acg cgc aac atc cgc gcg tcg       1152
Asp Phe Trp Gln Leu Gln Val Thr Thr Thr Arg Asn Ile Arg Ala Ser
    370                 375                 380
gta ttc atg gac tgg ttc acc ggt ggc ttg aac tac cag atc gac cat       1200
Val Phe Met Asp Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Asp His
385                 390                 395                 400
cac ctg ttc ccg ctc gtg ccg cgc cac aac ttg cca aag gtc aac gtg       1248
His Leu Phe Pro Leu Val Pro Arg His Asn Leu Pro Lys Val Asn Val
                405                 410                 415
ctc atc aag tcg cta tgc aag gag ttc gac atc ccg ttc cac gag acc       1296
Leu Ile Lys Ser Leu Cys Lys Glu Phe Asp Ile Pro Phe His Glu Thr
            420                 425                 430
ggc ttc tgg gag ggc atc tac gag gtc gtg gac cac ctg gcg gac atc       1344
Gly Phe Trp Glu Gly Ile Tyr Glu Val Val Asp His Leu Ala Asp Ile
        435                 440                 445
agc aag gaa ttc atc acc gag ttc cca gcg atg taa                       1380
Ser Lys Glu Phe Ile Thr Glu Phe Pro Ala Met
    450                 455
```

<210> 30
<211> 459
<212> PRT
<213> Pythium irregulare

<400> 30

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Met Val Asp Leu Lys Pro Gly Val Lys Arg Leu Val Ser Trp Lys Glu
1               5                  10                  15
Ile Arg Glu His Ala Thr Pro Ala Thr Ala Trp Ile Val Ile His His
            20                  25                  30
Lys Val Tyr Asp Ile Ser Lys Trp Asp Ser His Pro Gly Gly Ser Val
```

```
          35                  40                  45
Met Leu Thr Gln Ala Gly Glu Asp Ala Thr Asp Ala Phe Ala Val Phe
    50                  55                  60
His Pro Ser Ser Ala Leu Lys Leu Leu Glu Gln Phe Tyr Val Gly Asp
65                  70                  75                  80
Val Asp Glu Thr Ser Lys Ala Glu Ile Glu Gly Glu Pro Ala Ser Asp
                85                  90                  95
Glu Glu Arg Ala Arg Arg Glu Arg Ile Asn Glu Phe Ile Ala Ser Tyr
            100                 105                 110
Arg Arg Leu Arg Val Lys Val Lys Gly Met Gly Leu Tyr Asp Ala Ser
        115                 120                 125
Ala Leu Tyr Tyr Ala Trp Lys Leu Val Ser Thr Phe Gly Ile Ala Val
    130                 135                 140
Leu Ser Met Ala Ile Cys Phe Phe Phe Asn Ser Phe Ala Met Tyr Met
145                 150                 155                 160
Val Ala Gly Val Ile Met Gly Leu Phe Tyr Gln Gln Ser Gly Trp Leu
                165                 170                 175
Ala His Asp Phe Leu His Asn Gln Val Cys Glu Asn Arg Thr Leu Gly
            180                 185                 190
Asn Leu Ile Gly Cys Leu Val Gly Asn Ala Trp Gln Gly Phe Ser Val
        195                 200                 205
Gln Trp Trp Lys Asn Lys His Asn Leu His His Ala Val Pro Asn Leu
    210                 215                 220
His Ser Ala Lys Asp Glu Gly Phe Ile Gly Asp Pro Asp Ile Asp Thr
225                 230                 235                 240
Met Pro Leu Leu Ala Trp Ser Lys Glu Met Ala Arg Lys Ala Phe Glu
                245                 250                 255
Ser Ala His Gly Pro Phe Phe Ile Arg Asn Gln Ala Phe Leu Tyr Phe
            260                 265                 270
Pro Leu Leu Leu Leu Ala Arg Leu Ser Trp Leu Ala Gln Ser Phe Phe
        275                 280                 285
Tyr Val Phe Thr Glu Phe Ser Phe Gly Ile Phe Asp Lys Val Glu Phe
    290                 295                 300
Asp Gly Pro Glu Lys Ala Gly Leu Ile Val His Tyr Ile Trp Gln Leu
305                 310                 315                 320
Ala Ile Pro Tyr Phe Cys Asn Met Ser Leu Phe Glu Gly Val Ala Tyr
                325                 330                 335
Phe Leu Met Gly Gln Ala Ser Cys Gly Leu Leu Leu Ala Leu Val Phe
            340                 345                 350
Ser Ile Gly His Asn Gly Met Ser Val Tyr Glu Arg Glu Thr Lys Pro
        355                 360                 365
Asp Phe Trp Gln Leu Gln Val Thr Thr Thr Arg Asn Ile Arg Ala Ser
    370                 375                 380
Val Phe Met Asp Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Asp His
385                 390                 395                 400
His Leu Phe Pro Leu Val Pro Arg His Asn Leu Pro Lys Val Asn Val
                405                 410                 415
Leu Ile Lys Ser Leu Cys Lys Glu Phe Asp Ile Pro Phe His Glu Thr
            420                 425                 430
Gly Phe Trp Glu Gly Ile Tyr Glu Val Val Asp His Leu Ala Asp Ile
        435                 440                 445
Ser Lys Glu Phe Ile Thr Glu Phe Pro Ala Met
    450                 455

<210> 31
<211> 1374
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(1374)

<400> 31
atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag att ctg        48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                   10                  15
aat gcc gag gcc ctg aat gag ggc aag aag gat gcc gag gca cct ttc        96
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Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
                20                  25                  30
ttg atg atc atc gac aac aag gtg tat gat gtc cgc gag ttt gtc cct      144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
            35                  40                  45
gat cat ccc ggt gga agt gtt att ctc acg cac gtt ggc aag gac ggc      192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
        50                  55                  60
act gac gtc ttt gac act ttc cac ccc gag gct gct tgg gag aca ctt      240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tac gtt gga gat att gat gag agc gac cgt gcc atc aag      288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
aac gat gac ttt gcg gcc gag gtc cgc aag ctg cgt act ttg ttc cag      336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
tct ctg ggt tac tac gat tcc tcc aag gca tac tac gcc ttc aag gtc      384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
tca ttc aac ctc tgc atc tgg ggc ctg tcg acg ttc att gtt gcc aag      432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
agg ggc cag acc tcg acc ctc gcc aac gag ctc tcg gct gcg ctc ttg      480
Arg Gly Gln Thr Ser Thr Leu Ala Asn Glu Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctc ttc tgg cag cag aga gga tgg ttg gcg cac gac ttt ttg cac      528
Gly Leu Phe Trp Gln Gln Arg Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg gga gat ctt ttc ggc gcc ttc      576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
ttg gga gga gac tgc cag ggc ttc tcg tcc tca tgg tgg aag gac aag      624
Leu Gly Gly Asp Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
cac aac act cac cac gcc gcc ccc aac gtc cac ggc gag gat ccc gac      672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
att gac act cac cct ctg ttg acg tgg agt gag cat gct ttg gag atg      720
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
ttc tcg gac gtc cct gac gag gag ctg acc cgc atg tgg tcg cgc ttc      768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctt aac cag acc tgg ttc tac ttt ccc att ctc tcg ttt gcc      816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctc tcc tgg tgc ctc cag tcc atc ctc ttt gtt ctg cct aac ggt      864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
cag gcc cac aag ccc tct gga gcc cgt gtg ccc att tcc ttg gtc gag      912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
cag ctg tct ctt gcc atg cac tgg acc tgg tac ctc gcc acc atg ttc      960
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ttg ttc att aag gac ccc gtc aac atg atg gtg tac ttt ttg gtg tct     1008
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
cag gct gtt tgc ggt aac ctg ttg gcg att gtg ttc tcg ctc aac cac     1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggt atg cct gtg atc tcc aag gag gaa gcc gtc gac atg gat ttc     1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
ttc acc aag cag atc atc acg ggt cgt gat gtt cac cct ggt ctg ttc     1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
```

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gcc aac tgg ttc acg ggt gga ttg aac tac cag att gag cac cac ttg        1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc        1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag acc ttg tgc aaa aag tat ggt gtc cga tac cac acc act ggc atg        1296
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ttg aac gag gtc tcc aag        1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
gcc gcc tcc aag atg ggt aag gcg cag taa                                1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 32
<211> 457
<212> PRT
<213> Mortierella alpina

<400> 32
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
Arg Gly Gln Thr Ser Thr Leu Ala Asn Glu Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Arg Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Asp Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
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Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 33
<211> 1374
<212> DNA
<213> Mortierella isabellina

<220>
<221> CDS
<222> (1)..(1374)

<400> 33
atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag att ctg        48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
aat gcc gag gcc ctg aat gag ggc aag aag gat gcc gag gca cct ttc        96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
ttg atg atc atc gac aac aag gtg tat gat gtc cgc gag ttt gtc cct       144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
gat cat ccc ggt gga agt gtt att ctc acg cac gtt ggc aag gac ggc       192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
act gac gtc ttt gac act ttc cac ccc gag gct gct tgg gag aca ctt       240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tat gtt ggt gat att gat gag agc gac cgt gcc atc aaa       288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
aac gat gac ttt gcg gcc gag gtc cgc aag ctg cgt act ttg ttc cag       336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
tct ctg ggt tac tac gat tcc tcc aag gca tac tac gcc ttc aag gtc       384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
tca ttc aac ctc tgc atc tgg ggc ctg tcg acg ttc att gtt gcc aag       432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
tgg ggc cag acc tcg acc ctc gcc aac gta ctc tcg gct gcg ctc ttg       480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctc ttc tgg cag cag tgt gga tgg ttg gcg cac gac ttt ttg cac       528
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc       576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
ttg gga ggt gtc tgc cag ggc ttc tcg tcc tca tgg tgg aag gac aag       624
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
cac aac act cac cac gcc gcc ccc aac gtc cac ggc gag gat ccc gac       672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
att gac act cac cct ctg ttg acg tgg agt gag cat gct ttg gag atg       720
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
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ttc tcg gac gtc cct gac gag gag ctg acc cgc atg tgg tcg cgc ttc        768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctt aac cag acc tgg ttc tac ttt ccc att ctc tcg ttt gcc        816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctc tcc tgg tgc ctc cag tcc atc ctc ctt gtt ctg cct aac ggt        864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Leu Val Leu Pro Asn Gly
        275                 280                 285
cag gcc cac aag ccc tct gga gcc cgt gtg tcc att tcc ttg gtc gag        912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Ser Ile Ser Leu Val Glu
    290                 295                 300
cag ctg tct ctt gcc atg cac tgg acc tgg tac ctc gcc acc atg ttc        960
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ttg ttc att aag gac ccc gtc aac atg atg gtg tac ttt ttg gtg tct       1008
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
cag gct gtt tgc ggt aac ctg ttg gcg att gtg ttc tcg ctc aac cac       1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggt atg cct gtg atc tcc aag gag gaa gcc gtc gac atg gat ttc       1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
ttc acc aag cag atc atc acg ggt cgt gat gtt cac cct ggt ctg ttc       1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
gcc aat tgg ttc acg ggt gga ttg aac tac cag att gag cac cac ttg       1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc       1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag acc ttg tgc aaa aag tat ggt gtc cga tac cac acc act ggc atg       1296
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ttg aac gag gtc tcc aag       1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
gcc gcc tcc aag atg ggt aag gcg cag taa                              1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 34
<211> 457
<212> PRT
<213> Mortierella isabellina

<400> 34
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                  10                  15
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
```

```
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Leu Val Leu Pro Asn Gly
        275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Ser Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455
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<210> 35
<211> 1563
<212> DNA
<213> Ceratodon purpureus

<220>
<221> CDS
<222> (1)..(1563)

<400> 35

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atg gtg tcc cag ggc ggc ggt ctc tcg cag ggt tcc att gaa gaa aac        48
Met Val Ser Gln Gly Gly Gly Leu Ser Gln Gly Ser Ile Glu Glu Asn
1               5                  10                  15
att gac gtt gag cac ttg gca acg atg ccc ctc gtc agt gac ttc cta        96
Ile Asp Val Glu His Leu Ala Thr Met Pro Leu Val Ser Asp Phe Leu
            20                  25                  30
aat gtc ctg gga acg act ttg ggc cag tgg agt ctt tcc act aca ttc       144
Asn Val Leu Gly Thr Thr Leu Gly Gln Trp Ser Leu Ser Thr Thr Phe
        35                  40                  45
gct ttc aag agg ctc acg act aag aaa cac agt tcg gac atc tcg gtg       192
Ala Phe Lys Arg Leu Thr Thr Lys Lys His Ser Ser Asp Ile Ser Val
    50                  55                  60
gag gca caa aaa gaa tcg gtt gcg cgg ggg cca gtt gag aat att tct       240
Glu Ala Gln Lys Glu Ser Val Ala Arg Gly Pro Val Glu Asn Ile Ser
65                  70                  75                  80
caa tcg gtt gcg cag ccc atc agg cgg agg tgg gtg cag gat aaa aag       288
Gln Ser Val Ala Gln Pro Ile Arg Arg Arg Trp Val Gln Asp Lys Lys
                85                  90                  95
```

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ccg gtt act tac agc ctg aag gat gta gct tcg cac gat atg ccc cag          336
Pro Val Thr Tyr Ser Leu Lys Asp Val Ala Ser His Asp Met Pro Gln
                100                 105                 110
gac tgc tgg att ata atc aaa gag aag gtg tat gat gtg agc acc ttc          384
Asp Cys Trp Ile Ile Ile Lys Glu Lys Val Tyr Asp Val Ser Thr Phe
            115                 120                 125
gct gag cag cac cct gga ggc acg gtt atc aac acc tac ttc gga cga          432
Ala Glu Gln His Pro Gly Gly Thr Val Ile Asn Thr Tyr Phe Gly Arg
        130                 135                 140
gac gcc aca gat gtt ttc tct act ttc cac gca tcc acc tca tgg aag          480
Asp Ala Thr Asp Val Phe Ser Thr Phe His Ala Ser Thr Ser Trp Lys
145                 150                 155                 160
att ctt cag aat ttc tac atc ggg aac ctt gtt agg gag gag ccg act          528
Ile Leu Gln Asn Phe Tyr Ile Gly Asn Leu Val Arg Glu Glu Pro Thr
                165                 170                 175
ttg gag ctg ctg aag gag tac aga gag ttg aga gcc ctt ttc ttg aga          576
Leu Glu Leu Leu Lys Glu Tyr Arg Glu Leu Arg Ala Leu Phe Leu Arg
            180                 185                 190
gaa cag ctt ttc aag agt tcc aaa tcc tac tac ctt ttc aag act ctc          624
Glu Gln Leu Phe Lys Ser Ser Lys Ser Tyr Tyr Leu Phe Lys Thr Leu
        195                 200                 205
ata aat gtt tcc att gtt gcc aca agc att gcg ata atc agt ctg tac          672
Ile Asn Val Ser Ile Val Ala Thr Ser Ile Ala Ile Ile Ser Leu Tyr
    210                 215                 220
aag tct tac cgg gcg gtt ctg tta tca gcc agt ttg atg ggc ttg ttt          720
Lys Ser Tyr Arg Ala Val Leu Leu Ser Ala Ser Leu Met Gly Leu Phe
225                 230                 235                 240
att caa cag tgc gga tgg ttg tct cac gat ttt cta cac cat cag gta          768
Ile Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val
                245                 250                 255
ttt gag aca cgc tgg ctc aat gac gtt gtt ggc tat gtg gtc ggc aac          816
Phe Glu Thr Arg Trp Leu Asn Asp Val Val Gly Tyr Val Val Gly Asn
            260                 265                 270
gtt gtt ctg gga ttc agt gtc tcg tgg tgg aag acc aag cac aac ctg          864
Val Val Leu Gly Phe Ser Val Ser Trp Trp Lys Thr Lys His Asn Leu
        275                 280                 285
cat cat gct gct ccg aat gaa tgc gac caa aag tac aca ccg att gat          912
His His Ala Ala Pro Asn Glu Cys Asp Gln Lys Tyr Thr Pro Ile Asp
    290                 295                 300
gag gat att gat act ctc ccc atc att gct tgg agt aaa gat ctc ttg          960
Glu Asp Ile Asp Thr Leu Pro Ile Ile Ala Trp Ser Lys Asp Leu Leu
305                 310                 315                 320
gcc act gtt gag agc aag acc atg ttg cga gtt ctt cag tac cag cac         1008
Ala Thr Val Glu Ser Lys Thr Met Leu Arg Val Leu Gln Tyr Gln His
                325                 330                 335
cta ttc ttt ttg gtt ctt ttg acg ttt gcc cgg gcg agt tgg cta ttt         1056
Leu Phe Phe Leu Val Leu Leu Thr Phe Ala Arg Ala Ser Trp Leu Phe
            340                 345                 350
tgg agc gcg gcc ttc act ctc agg ccc gag ttg acc ctt ggc gag aag         1104
Trp Ser Ala Ala Phe Thr Leu Arg Pro Glu Leu Thr Leu Gly Glu Lys
        355                 360                 365
ctt ttg gag agg gga acg atg gct ttg cac tac att tgg ttt aat agt         1152
Leu Leu Glu Arg Gly Thr Met Ala Leu His Tyr Ile Trp Phe Asn Ser
    370                 375                 380
gtt gcg ttt tat ctg ctc ccc gga tgg aaa cca gtt gta tgg atg gtg         1200
Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
385                 390                 395                 400
gtc agc gag ctc atg tct ggt ttc ctg ctg gga tac gta ttt gta ctc         1248
Val Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
                405                 410                 415
agt cac aat gga atg gag gtg tac aat acg tca aag gac ttc gtg aat         1296
Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
            420                 425                 430
gcc cag att gca tcg act cgc gac atc aaa gca ggg gtg ttt aat gat         1344
Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
        435                 440                 445
tgg ttc acc gga ggt ctc aac aga cag att gag cat cat cta ttt cca         1392
Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro
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    450                 455                 460
acg atg ccc agg cac aac ctt aat aaa att tct cct cac gtg gag act        1440
Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
465                 470                 475                 480
ttg tgc aag aag cat gga ctg gtc tac gaa gac gtg agc atg gct tcg        1488
Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
                485                 490                 495
ggc act tac cgg gtt ttg aaa aca ctt aag gac gtt gcc gat gct gct        1536
Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
            500                 505                 510
tca cac cag cag ctt gct gcg agt tga                                   1563
Ser His Gln Gln Leu Ala Ala Ser
        515                 520

<210> 36
<211> 520
<212> PRT
<213> Ceratodon purpureus

<400> 36
Met Val Ser Gln Gly Gly Gly Leu Ser Gln Gly Ser Ile Glu Glu Asn
1               5                   10                  15
Ile Asp Val Glu His Leu Ala Thr Met Pro Leu Val Ser Asp Phe Leu
            20                  25                  30
Asn Val Leu Gly Thr Thr Leu Gly Gln Trp Ser Leu Ser Thr Thr Phe
        35                  40                  45
Ala Phe Lys Arg Leu Thr Thr Lys Lys His Ser Ser Asp Ile Ser Val
    50                  55                  60
Glu Ala Gln Lys Glu Ser Val Ala Arg Gly Pro Val Glu Asn Ile Ser
65                  70                  75                  80
Gln Ser Val Ala Gln Pro Ile Arg Arg Arg Trp Val Gln Asp Lys Lys
                85                  90                  95
Pro Val Thr Tyr Ser Leu Lys Asp Val Ala Ser His Asp Met Pro Gln
            100                 105                 110
Asp Cys Trp Ile Ile Ile Lys Glu Lys Val Tyr Asp Val Ser Thr Phe
        115                 120                 125
Ala Glu Gln His Pro Gly Gly Thr Val Ile Asn Thr Tyr Phe Gly Arg
    130                 135                 140
Asp Ala Thr Asp Val Phe Ser Thr Phe His Ala Ser Thr Ser Trp Lys
145                 150                 155                 160
Ile Leu Gln Asn Phe Tyr Ile Gly Asn Leu Val Arg Glu Glu Pro Thr
                165                 170                 175
Leu Glu Leu Leu Lys Glu Tyr Arg Glu Leu Arg Ala Leu Phe Leu Arg
            180                 185                 190
Glu Gln Leu Phe Lys Ser Ser Lys Ser Tyr Tyr Leu Phe Lys Thr Leu
        195                 200                 205
Ile Asn Val Ser Ile Val Ala Thr Ser Ile Ala Ile Ile Ser Leu Tyr
    210                 215                 220
Lys Ser Tyr Arg Ala Val Leu Leu Ser Ala Ser Leu Met Gly Leu Phe
225                 230                 235                 240
Ile Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val
                245                 250                 255
Phe Glu Thr Arg Trp Leu Asn Asp Val Val Gly Tyr Val Val Gly Asn
            260                 265                 270
Val Val Leu Gly Phe Ser Val Ser Trp Trp Lys Thr Lys His Asn Leu
        275                 280                 285
His His Ala Ala Pro Asn Glu Cys Asp Gln Lys Tyr Thr Pro Ile Asp
    290                 295                 300
Glu Asp Ile Asp Thr Leu Pro Ile Ile Ala Trp Ser Lys Asp Leu Leu
305                 310                 315                 320
Ala Thr Val Glu Ser Lys Thr Met Leu Arg Val Leu Gln Tyr Gln His
                325                 330                 335
Leu Phe Phe Leu Val Leu Leu Thr Phe Ala Arg Ala Ser Trp Leu Phe
            340                 345                 350
Trp Ser Ala Ala Phe Thr Leu Arg Pro Glu Leu Thr Leu Gly Glu Lys
        355                 360                 365
Leu Leu Glu Arg Gly Thr Met Ala Leu His Tyr Ile Trp Phe Asn Ser
    370                 375                 380
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Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
385                 390                 395                 400
Val Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
                405                 410                 415
Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
            420                 425                 430
Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
        435                 440                 445
Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro
    450                 455                 460
Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
465                 470                 475                 480
Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
                485                 490                 495
Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
            500                 505                 510
Ser His Gln Gln Leu Ala Ala Ser
        515                 520

<210> 37
<211> 1452
<212> DNA
<213> Ceratodon purpureus

<220>
<221> CDS
<222> (1)..(1452)

<400> 37
atg gcc ctc gtt acc gac ttt ctg aac ttt ctg ggc acg aca tgg agc        48
Met Ala Leu Val Thr Asp Phe Leu Asn Phe Leu Gly Thr Thr Trp Ser
1               5                  10                  15
aag tac agc gtg tac acc cat agc tat gct gga aac tat ggg cct act        96
Lys Tyr Ser Val Tyr Thr His Ser Tyr Ala Gly Asn Tyr Gly Pro Thr
            20                  25                  30
ttg aag cac gcc aaa aag gtt tct gct caa ggt aaa act gcg gga cag       144
Leu Lys His Ala Lys Lys Val Ser Ala Gln Gly Lys Thr Ala Gly Gln
        35                  40                  45
aca ctg aga cag aga tcg gtg cag gac aaa aag cca ggc act tac tct       192
Thr Leu Arg Gln Arg Ser Val Gln Asp Lys Lys Pro Gly Thr Tyr Ser
    50                  55                  60
ctg gcc gat gtt gct tct cac gac agg cct gga gac tgc tgg atg atc       240
Leu Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp Met Ile
65                  70                  75                  80
gtc aaa gag aag gtg tat gat att agc cgt ttt gcg gac gac cac cct       288
Val Lys Glu Lys Val Tyr Asp Ile Ser Arg Phe Ala Asp Asp His Pro
                85                  90                  95
gga ggg acg gta att agc acc tac ttt ggg cgg gat ggc aca gac gtt       336
Gly Gly Thr Val Ile Ser Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val
            100                 105                 110
ttc gca aca ttc cat cca cct gcc gca tgg aag caa ctc aat gac tac       384
Phe Ala Thr Phe His Pro Pro Ala Ala Trp Lys Gln Leu Asn Asp Tyr
        115                 120                 125
tac att gga gac ctt gct agg gaa gag ccc ctt gat gaa ttg ctt aaa       432
Tyr Ile Gly Asp Leu Ala Arg Glu Glu Pro Leu Asp Glu Leu Leu Lys
    130                 135                 140
gac tac aga gat atg aga gcc gag ttt gtt aga gaa ggg ctt ttc aag       480
Asp Tyr Arg Asp Met Arg Ala Glu Phe Val Arg Glu Gly Leu Phe Lys
145                 150                 155                 160
agt tcc aag gcc tgg ttc ctg ctt cag act ctg att aat gca gct ctc       528
Ser Ser Lys Ala Trp Phe Leu Leu Gln Thr Leu Ile Asn Ala Ala Leu
                165                 170                 175
ttt gct gcg agc att gcg act atc tgt tac gac aag agt tac tgg gct       576
Phe Ala Ala Ser Ile Ala Thr Ile Cys Tyr Asp Lys Ser Tyr Trp Ala
            180                 185                 190
att gtg ctg tca gcc agt ttg atg ggt ctc ttc gtc caa cag tgt gga       624
Ile Val Leu Ser Ala Ser Leu Met Gly Leu Phe Val Gln Gln Cys Gly
        195                 200                 205
```

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tgg ctt gcc cat gat ttc ctt cat caa cag gtc ttt gag aac cgt acc        672
Trp Leu Ala His Asp Phe Leu His Gln Gln Val Phe Glu Asn Arg Thr
    210                 215                 220
gcg aac tcc ttc ttt ggc tat ttg ttc ggc aat tgc gtg ctt ggc ttt        720
Ala Asn Ser Phe Phe Gly Tyr Leu Phe Gly Asn Cys Val Leu Gly Phe
225                 230                 235                 240
agt gta tca tgg tgg agg acg aag cac aac att cat cat act gct ccg        768
Ser Val Ser Trp Trp Arg Thr Lys His Asn Ile His His Thr Ala Pro
                245                 250                 255
aat gag tgc gac gaa cag tac aca cct cta gac gaa gac att gat act        816
Asn Glu Cys Asp Glu Gln Tyr Thr Pro Leu Asp Glu Asp Ile Asp Thr
            260                 265                 270
ctc ccc atc att gcc tgg agc aag gaa att ttg gcc acc gtt gag agc        864
Leu Pro Ile Ile Ala Trp Ser Lys Glu Ile Leu Ala Thr Val Glu Ser
        275                 280                 285
aag aga att ttg cga gtg ctt caa tat cag cac tac atg att ctg cct        912
Lys Arg Ile Leu Arg Val Leu Gln Tyr Gln His Tyr Met Ile Leu Pro
    290                 295                 300
cta ttg ttc atg gcc cgg tac agt tgg act ttt gga agt ttg ctc ttc        960
Leu Leu Phe Met Ala Arg Tyr Ser Trp Thr Phe Gly Ser Leu Leu Phe
305                 310                 315                 320
aca ttc aat cct gat ttg agc acg acc aag gga ttg ata gag aag gga       1008
Thr Phe Asn Pro Asp Leu Ser Thr Thr Lys Gly Leu Ile Glu Lys Gly
                325                 330                 335
aca gtt gct ttt cac tac gcc tgg ttc agt tgg gct gcg ttc cat att       1056
Thr Val Ala Phe His Tyr Ala Trp Phe Ser Trp Ala Ala Phe His Ile
            340                 345                 350
ttg ccg ggt gtc gct aag cct ctt gcg tgg atg gta gca act gag ctt       1104
Leu Pro Gly Val Ala Lys Pro Leu Ala Trp Met Val Ala Thr Glu Leu
        355                 360                 365
gtg gcc ggt ttg ttg ttg gga ttc gtg ttt acg ttg agt cac aat gga       1152
Val Ala Gly Leu Leu Leu Gly Phe Val Phe Thr Leu Ser His Asn Gly
    370                 375                 380
aag gag gtt tac aat gaa tcg aag gac ttc gtg aga gcc cag gtt att       1200
Lys Glu Val Tyr Asn Glu Ser Lys Asp Phe Val Arg Ala Gln Val Ile
385                 390                 395                 400
acc acc cgt aac acc aag cga ggc tgg ttc aac gat tgg ttc act ggg       1248
Thr Thr Arg Asn Thr Lys Arg Gly Trp Phe Asn Asp Trp Phe Thr Gly
                405                 410                 415
gga ctc gac acc cag att gag cat cac ctg ttt cca aca atg ccc agg       1296
Gly Leu Asp Thr Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg
            420                 425                 430
cac aac tac ccc aag atc gca cct cag gtc gag gct ctt tgc aag aag       1344
His Asn Tyr Pro Lys Ile Ala Pro Gln Val Glu Ala Leu Cys Lys Lys
        435                 440                 445
cac ggc ctc gag tac gat aat gtc tcc gtc gtt ggt gcc tct gtc gcg       1392
His Gly Leu Glu Tyr Asp Asn Val Ser Val Val Gly Ala Ser Val Ala
    450                 455                 460
gtt gtg aag gcg ctc aag gaa att gct gat gaa gcg tca att cgg ctt       1440
Val Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile Arg Leu
465                 470                 475                 480
cac gct cac taa                                                      1452
His Ala His
```

<210> 38
<211> 483
<212> PRT
<213> Ceratodon purpureus

<400> 38

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Met Ala Leu Val Thr Asp Phe Leu Asn Phe Leu Gly Thr Thr Trp Ser
1               5                  10                  15
Lys Tyr Ser Val Tyr Thr His Ser Tyr Ala Gly Asn Tyr Gly Pro Thr
            20                  25                  30
Leu Lys His Ala Lys Lys Val Ser Ala Gln Gly Lys Thr Ala Gly Gln
        35                  40                  45
Thr Leu Arg Gln Arg Ser Val Gln Asp Lys Lys Pro Gly Thr Tyr Ser
```

```
     50                  55                  60
Leu Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp Met Ile
65                  70                  75                  80
Val Lys Glu Lys Val Tyr Asp Ile Ser Arg Phe Ala Asp Asp His Pro
                85                  90                  95
Gly Gly Thr Val Ile Ser Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val
            100                 105                 110
Phe Ala Thr Phe His Pro Pro Ala Ala Trp Lys Gln Leu Asn Asp Tyr
        115                 120                 125
Tyr Ile Gly Asp Leu Ala Arg Glu Glu Pro Leu Asp Glu Leu Leu Lys
    130                 135                 140
Asp Tyr Arg Asp Met Arg Ala Glu Phe Val Arg Glu Gly Leu Phe Lys
145                 150                 155                 160
Ser Ser Lys Ala Trp Phe Leu Leu Gln Thr Leu Ile Asn Ala Ala Leu
                165                 170                 175
Phe Ala Ala Ser Ile Ala Thr Ile Cys Tyr Asp Lys Ser Tyr Trp Ala
            180                 185                 190
Ile Val Leu Ser Ala Ser Leu Met Gly Leu Phe Val Gln Gln Cys Gly
        195                 200                 205
Trp Leu Ala His Asp Phe Leu His Gln Gln Val Phe Glu Asn Arg Thr
    210                 215                 220
Ala Asn Ser Phe Phe Gly Tyr Leu Phe Gly Asn Cys Val Leu Gly Phe
225                 230                 235                 240
Ser Val Ser Trp Trp Arg Thr Lys His Asn Ile His His Thr Ala Pro
                245                 250                 255
Asn Glu Cys Asp Glu Gln Tyr Thr Pro Leu Asp Glu Asp Ile Asp Thr
            260                 265                 270
Leu Pro Ile Ile Ala Trp Ser Lys Glu Ile Leu Ala Thr Val Glu Ser
        275                 280                 285
Lys Arg Ile Leu Arg Val Leu Gln Tyr Gln His Tyr Met Ile Leu Pro
    290                 295                 300
Leu Leu Phe Met Ala Arg Tyr Ser Trp Thr Phe Gly Ser Leu Leu Phe
305                 310                 315                 320
Thr Phe Asn Pro Asp Leu Ser Thr Thr Lys Gly Leu Ile Glu Lys Gly
                325                 330                 335
Thr Val Ala Phe His Tyr Ala Trp Phe Ser Trp Ala Ala Phe His Ile
            340                 345                 350
Leu Pro Gly Val Ala Lys Pro Leu Ala Trp Met Val Ala Thr Glu Leu
        355                 360                 365
Val Ala Gly Leu Leu Leu Gly Phe Val Phe Thr Leu Ser His Asn Gly
    370                 375                 380
Lys Glu Val Tyr Asn Glu Ser Lys Asp Phe Val Arg Ala Gln Val Ile
385                 390                 395                 400
Thr Thr Arg Asn Thr Lys Arg Gly Trp Phe Asn Asp Trp Phe Thr Gly
                405                 410                 415
Gly Leu Asp Thr Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg
            420                 425                 430
His Asn Tyr Pro Lys Ile Ala Pro Gln Val Glu Ala Leu Cys Lys Lys
        435                 440                 445
His Gly Leu Glu Tyr Asp Asn Val Ser Val Val Gly Ala Ser Val Ala
    450                 455                 460
Val Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile Arg Leu
465                 470                 475                 480
His Ala His
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<210> 39
<211> 1374
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(1374)

<400> 39

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Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Val Leu
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1                   5                  10                  15
aat gcc gag gct ctg aat gag ggc aag aag gat gcc gag gca ccc ttc          96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
ttg atg atc atc gac aac aag gtg tac gat gtc cgc gag ttc gtc cct         144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
gat cat ccc ggt gga agt gtg att ctc acg cac gtt ggc aag gac ggc         192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
act gac gtc ttt gac act ttt cac ccc gag gct gct tgg gag act ctt         240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tac gtt ggt gat att gac gag agc gac cgc gat atc aag         288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
                85                  90                  95
aat gat gac ttt gcg gcc gag gtc cgc aag ctg cgt acc ttg ttc cag         336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
tct ctt ggt tac tac gat tct tcc aag gca tac tac gcc ttc aag gtc         384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
tcg ttc aac ctc tgc atc tgg ggt ttg tcg acg gtc att gtg gcc aag         432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
    130                 135                 140
tgg ggc cag acc tcg acc ctc gcc aac gtg ctc tcg gct gcg ctt ttg         480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctg ttc tgg cag cag tgc gga tgg ttg gct cac gac ttt ttg cat         528
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc         576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
ttg gga ggt gtc tgc cag ggc ttc tcg tcc tcg tgg tgg aag gac aag         624
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
cac aac act cac cac gcc gcc ccc aac gtc cac ggc gag gat ccc gac         672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
att gac acc cac cct ctg ttg acc tgg agt gag cat gcg ttg gag atg         720
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
ttc tcg gat gtc cca gat gag gag ctg acc cgc atg tgg tcg cgt ttc         768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctg aac cag acc tgg ttt tac ttc ccc att ctc tcg ttt gcc         816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctc tcc tgg tgc ctc cag tcc att ctc ttt gtg ctg cct aac ggt         864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
cag gcc cac aag ccc tcg ggc gcg cgt gtg ccc atc tcg ttg gtc gag         912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
cag ctg tcg ctt gcg atg cac tgg acc tgg tac ctc gcc acc atg ttc         960
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ctg ttc atc aag gat ccc gtc aac atg ctg gtg tac ttt ttg gtg tcg        1008
Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
                325                 330                 335
cag gcg gtg tgc gga aac ttg ttg gcg atc gtg ttc tcg ctc aac cac        1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggt atg cct gtg atc tcg aag gag gag gcg gtc gat atg gat ttc        1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
ttc acg aag cag atc atc acg ggt cgt gat gtc cac ccg ggt cta ttt        1152
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Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
gcc aac tgg ttc acg ggt gga ttg aac tat cag atc gag cac cac ttg      1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc      1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag acc ctg tgc aaa aag tac aat gtc cga tac cac acc acc ggt atg      1296
Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ctg aac gag gtc tcc aag      1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
gct gcc tcc aag atg ggt aag gcg cag taa                             1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 40
<211> 457
<212> PRT
<213> Mortierella alpina

<400> 40
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Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
    130                 135                 140
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
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Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
            355                 360                 365
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
            435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 41
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<212> DNA
<213> Mortierella alpina

<220>
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<222> (1)..(1374)

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atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag att ttg        48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Ile Leu
1               5                   10                  15
aat gcc gag gcc ctg aat gag ggc aag aag gat gcc gag gca ccc ttt        96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
ctg atg atc att gac aac aag gtg tac gat gtc cgc gag ttt gtc cct       144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
gat cat ccc ggt gga agt gtg att ctc acg cac gtt ggc aag gac ggc       192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
act gac gtc ttt gac act ttc cac ccc gag gct gct tgg gag act ctt       240
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
gcc aac ttt tac gtt ggt gat att gat gag agc gat cgt gcc atc aag       288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
aat gat gac ttt gcg gcc gag gtt cgc aag ctg cgc acc ttg ttc cag       336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
tcc ctt ggc tac tac gac tcg tcc aag gca tac tat gcc ttc aag gtc       384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
tcg ttc aac ctc tgc atc tgg ggc ttg tcg act ttc att gtt gcc aag       432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
    130                 135                 140
tgg ggc cag acc tcg acc ctc gcc aac gtg ctc tcg gct gcg ctc ttg       480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
ggt ctc ttc tgg cag cag tgc gga tgg ttg gcg cac gac ttt ttg cac       528
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc       576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
ttg gga ggt gtc tgc cag ggt ttc tcg tcc tcc tgg tgg aag gac aag       624
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
cac aac act cac cac gct gct ccc aac gtc cac ggc gag gat ccc gac       672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
att gac act cac cct ctg ttg acc tgg agt gag cat gct ctg gag atg       720
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Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
ttc tcg gat gtt cct gac gag gag ctg acc cgt atg tgg tcg cgc ttc        768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
atg gtc ctc aac cag acc tgg ttc tac ttc ccc att ctc tcg ttt gcc        816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
cgt ctg tcc tgg tgc ctc cag tcc att atg ttt gtt ctg ccc aac ggt        864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Met Phe Val Leu Pro Asn Gly
        275                 280                 285
cag gcc cac aag ccc tct gga gcg cgt gtg ccc att tcg ttg gtc gag        912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
cag ctg tct ctg gct atg cac tgg acc tgg tac ctc gcc acc atg ttc        960
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
ctg ttc att aag gat ccc gtc aac atg att gtg tac ttt ttg gtg tcg       1008
Leu Phe Ile Lys Asp Pro Val Asn Met Ile Val Tyr Phe Leu Val Ser
                325                 330                 335
cag gct gtt tgc ggc aac ttg ttg gcg att gtg ttc tcg ctc aac cac       1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
aac ggc atg cct gtg atc tcc aag gag gaa gcg gtc gat atg gac ttc       1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
ttc acc aag cag atc atc acg ggt cgt gat gtt cac cct ggt ctg ttt       1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
gcc aac tgg ttc acg ggt gga ttg aac tac cag att gag cac cac ttg       1200
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
ttc cct tcg atg ccc cgc cac aac ttt tca aag atc cag cct gct gtc       1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
gag act ttg tgc aaa aag tac ggt gtc cga tac cat acc act ggt atg       1296
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
atc gag gga act gca gag gtc ttt agc cgt ttg aac gag gtc tcc aag       1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
gcg gcc tcc aag atg ggc aag gca cag taa                              1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455

<210> 42
<211> 457
<212> PRT
<213> Mortierella alpina

<400> 42
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Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Ala Ile Lys
                85                  90                  95
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Ala Lys
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    130                 135                 140
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270
Arg Leu Ser Trp Cys Leu Gln Ser Ile Met Phe Val Leu Pro Asn Gly
        275                 280                 285
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320
Leu Phe Ile Lys Asp Pro Val Asn Met Ile Val Tyr Phe Leu Val Ser
                325                 330                 335
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415
Glu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455


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<211> 444
<212> PRT
<213> Artificial sequence

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<223> xaa in position 11 is any or no amino acid
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<223> xaa in position 17 is any or no amino acid
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<222> (19)..(20)
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<223> xaa in position 153 is any amino acid
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<222> (174)..(176)
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<223> xaa in position 201 to 207 is any or no amino acid

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<223> xaa in position 285 to 296 is any or no amino acid
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<222> (298)..(298)
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<222> (300)..(305)
<223> xaa in position 300 to 305 is any amino acid
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<221> Variant
<222> (307)..(326)
<223> xaa in position 307 to 326 is any amino acid
<220>
<221> Variant
<222> (327)..(328)
<223> xaa in position 327 to 328 is any or no amino acid
<220>
<221> Variant
<222> (330)..(336)
<223> xaa in position 330 to 336 is any amino acid

<220>
<221> Variant
<222> (338)..(339)
<223> xaa in position 338 to 339 is any amino acid
<220>
<221> Variant
<222> (342)..(342)
<223> xaa in position 342 is any amino acid
<220>
<221> Variant
<222> (345)..(347)
<223> xaa in position 345 to 347 is any amino acid
<220>
<221> Variant
<222> (352)..(352)
<223> xaa in position 352 is any amino acid
<220>
<221> Variant
<222> (354)..(359)
<223> xaa in position 354 to 359 is any amino acid
<220>
<221> Variant
<222> (360)..(363)
<223> xaa in position 360 to 363 is any or no amino acid
<220>
<221> Variant
<222> (365)..(367)
<223> xaa in position 365 to 367 is any amino acid
<220>
<221> Variant
<222> (369)..(370)
<223> xaa in position 369 to 370 is any amino acid
<220>
<221> Variant
<222> (372)..(372)
<223> xaa in position 372 is any amino acid
<220>
<221> Variant
<222> (374)..(379)
<223> xaa in position 374 to 379 is any amino acid
<220>
<221> Variant
<222> (380)..(384)
<223> xaa in position 380 to 384 is any or no amino acid
<220>
<221> Variant
<222> (386)..(387)
<223> xaa in position 386 to 387 is any amino acid
<220>
<221> Variant
<222> (390)..(390)
<223> xaa in position 390 is any amino acid
<220>
<221> Variant
<222> (404)..(405)
<223> xaa in position 404 to 405 is any amino acid
<220>
<221> Variant
<222> (410)..(411)
<223> xaa in position 410 to 411 is any amino acid
<220>
<221> Variant
<222> (413)..(416)
<223> xaa in position 413 to 416 is any amino acid
<220>
<221> Variant
<222> (418)..(419)
<223> xaa in position 418 to 419 is any amino acid

<220>
<221> Variant
<222> (423)..(427)
<223> xaa in position 423 to 427 is any amino acid
<220>
<221> Variant
<222> (430)..(435)
<223> xaa in position 430 to 435 is any amino acid
<220>
<221> Variant
<222> (438)..(438)
<223> xaa in position 438 is any amino acid
<220>
<221> Variant
<222> (441)..(443)
<223> xaa in position 441 to 443 is any amino acid

<400> 43
Lys Val Tyr Asp xaa xaa xaa xaa xaa xaa xaa His Pro Gly Gly xaa
1 5 10 15
xaa Val xaa xaa Thr xaa xaa Gly xaa Asp xaa Thr Asp xaa Phe xaa
20 25 30
xaa xaa His Pro xaa xaa Ala xaa xaa xaa Leu xaa xaa xaa Tyr xaa
35 40 45
Gly Asp xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
50 55 60
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Phe xaa xaa
65 70 75 80
xaa xaa Arg xaa Leu Arg xaa xaa xaa xaa xaa xaa Gly xaa xaa xaa
85 90 95
xaa Ser xaa xaa xaa Tyr xaa xaa Lys xaa xaa xaa xaa xaa xaa xaa
100 105 110
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
115 120 125
xaa xaa xaa xaa xaa xaa Ala xaa xaa xaa Gly xaa Phe xaa Gln Gln
130 135 140
xaa Gly Trp Leu Ala His Asp Phe xaa His His Gln Val Phe xaa xaa
145 150 155 160
Arg xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa xaa xaa
165 170 175
Gln Gly Phe Ser xaa xaa Trp Trp Lys xaa Lys His Asn xaa His His
180 185 190
Ala xaa xaa Asn xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Asp
195 200 205
Pro Asp Ile Asp Thr xaa Pro xaa Leu xaa Trp xaa xaa xaa xaa xaa
210 215 220
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
225 230 235 240
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Gln xaa xaa xaa xaa Phe
245 250 255
xaa xaa Leu xaa xaa Ala Arg xaa Ser Trp xaa xaa xaa Ser xaa xaa
260 265 270
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
275 280 285
xaa xaa xaa xaa xaa xaa xaa xaa Leu xaa Glu xaa xaa xaa xaa xaa
290 295 300
xaa His xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
305 310 315 320
xaa xaa xaa xaa xaa xaa xaa xaa Phe xaa xaa xaa xaa xaa xaa xaa
325 330 335
Gly xaa xaa Leu Ala xaa Val Phe xaa xaa xaa His Asn Gly Met xaa
340 345 350
Val xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Phe xaa xaa xaa Gln
355 360 365
xaa xaa Thr xaa Arg xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
370 375 380
Phe xaa xaa Trp Phe xaa Gly Gly Leu Asn Tyr Gln Ile Glu His His
385 390 395 400

```
Leu Phe Pro xaa xaa Pro Arg His Asn xaa xaa Lys xaa xaa xaa xaa
                405                 410                 415
Val xaa xaa Leu Cys Lys xaa xaa xaa xaa xaa Tyr His xaa xaa xaa
            420                 425                 430
xaa xaa xaa Gly Thr xaa Glu Val xaa xaa xaa Leu
        435                 440
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<210> 44
<211> 31
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Asp or Glu
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Leu or Val
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Leu, Met or Val
<220>
<221> Variant
<222> (17)..(18)
<223> xaa in position 17 to 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Ala, Asn, Pro or Ser
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ile or Val
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is any amino acid
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Pro or Val
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is any amino acid
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is Ile or Val
<220>
<221> Variant
<222> (26)..(26)

<223> xaa in position 26 is Glu or Lys
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Ala, Ser or Thr
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is any or no amino acid

<400> 44
Leu xaa xaa Gln Ile xaa His His xaa Phe Pro xaa xaa Pro Arg His
  1   5   10   15
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Leu Cys xaa Lys
            20   25   30

<210> 45
<211> 27
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Asp, Gly or Asn
<220>
<221> Variant
<222> (3)..(4)
<223> xaa in position 3 to 4 is any amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is any amino acid
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is Ala, Gln or Ser
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Asp or Asn
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ala or Ser
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ala, Ser or Val
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Pro or Thr
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is Leu or Val
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is any amino acid
<220>
<221> Variant

<222> (27)..(27)
<223> xaa in position 27 is Glu, Gly, Asn or Ser

<400> 45
Gly xaa xaa xaa Gln Gly Phe Ser xaa xaa Trp Trp Lys xaa Lys His
  1   5   10   15
Asn xaa His His xaa xaa xaa Asn xaa xaa xaa
            20   25

<210> 46
<211> 31
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Glu, Lys or Arg
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Ala or Gly
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Ser or Thr
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is Ala, Ser or Val
<220>
<221> Variant
<222> (12)..(13)
<223> xaa in position 12 to 13 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Phe or Met
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Pro or Ser
<220>
<221> Variant
<222> (17)..(18)
<223> xaa in position 17 to 18 is any amino acid
<220>
<221> Variant
<222> (20)..(22)
<223> xaa in position 20 to 22 is any amino acid
<220>
<221> Variant
<222> (24)..(25)
<223> xaa in position 24 to 25 is any amino acid
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is any or no amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is any amino acid
<220>

<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is any or no amino acid

<400> 46
Thr xaa xaa Gly xaa Asp xaa xaa Asp xaa Phe xaa xaa xaa His xaa
  1   5   10   15
xaa xaa Ala xaa xaa Leu xaa xaa xaa Tyr xaa xaa Gly Asp
            20   25   30

<210> 47
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Phe, Leu or Val
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is any amino acid
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Ala, Gly or Ser
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Gln or Thr
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Ala, Cys or Pro
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Ser, Thr or Val
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Cys, Ser or Thr
<220>
<221> Variant
<222> (10)..(11)
<223> xaa in position 10 to 11 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Ile or Leu
<220>
<221> Variant
<222> (17)..(18)
<223> xaa in position 17 to 18 is any amino acid
<220>
<221> Variant
<222> (19)..(20)
<223> xaa in position 19 to 20 is any or no amino acid
<220>
<221> Variant
<222> (22)..(23)
<223> xaa in position 22 to 23 is any or no amino acid
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is Ala, Pro or Ser

<400> 47
Phe xaa xaa xaa xaa xaa xaa xaa Gly xaa xaa Leu Ala xaa Val Phe
1 5 10 15
xaa xaa xaa xaa Asn xaa xaa Gly Met xaa
20 25

<210> 48
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any amino acid
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is any or no amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is any or no amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Asp or Ser

<400> 48
Pro Asp Ile Asp Thr xaa Pro xaa Leu Leu xaa Trp xaa
1 5 10

<210> 49
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is any or no amino acid
<220>
<221> Variant
<222> (7)..(8)
<223> xaa in position 7 to 8 is any amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is any or no amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is any amino acid
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Phe or Leu
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ile or Leu

<400> 49
Gln xaa xaa xaa Tyr Phe xaa xaa xaa Leu xaa xaa Ala Arg xaa Ser
1 5 10 15
Trp

<210> 50
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Asp, Glu or Thr
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is Ala or Thr
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Asp, Gly, Ser or Thr
<220>
<221> Variant
<222> (5)..(7)
<223> xaa in position 5 to 7 is any amino acid
<220>
<221> Variant
<222> (9)..(10)
<223> xaa in position 9 to 10 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Phe, Leu or Val
<220>
<221> Variant
<222> (14)..(15)
<223> xaa in position 14 to 15 is any amino acid
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Ala, Asp, Gly, Asn or Ser
<220>
<221> Variant
<222> (18)..(19)
<223> xaa in position 18 to 19 is any amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Ala, Gln or Ser

<400> 50
His xaa xaa xaa xaa xaa xaa Gly xaa xaa Glu Val xaa xaa xaa Leu
1 5 10 15
xaa xaa xaa xaa
20

<210> 51
<211> 1320
<212> DNA
<213> Thraustochytrium ssp.

<220>
<221> CDS
<222> (1)..(1320)

<400> 51

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atg ggc aag ggc agc gag ggc cgc agc gcg gcg cgc gag atg acg gcc        48
Met Gly Lys Gly Ser Glu Gly Arg Ser Ala Ala Arg Glu Met Thr Ala
1               5                  10                  15
gag gcg aac ggc gac aag cgg aaa acg att ctg atc gag ggc gtc ctg        96
Glu Ala Asn Gly Asp Lys Arg Lys Thr Ile Leu Ile Glu Gly Val Leu
            20                  25                  30
tac gac gcg acg aac ttt aag cac ccg ggc ggt tcg atc atc aac ttc       144
Tyr Asp Ala Thr Asn Phe Lys His Pro Gly Gly Ser Ile Ile Asn Phe
        35                  40                  45
ttg acc gag ggc gag gcc ggc gtg gac gcg acg cag gcg tac cgc gag       192
Leu Thr Glu Gly Glu Ala Gly Val Asp Ala Thr Gln Ala Tyr Arg Glu
    50                  55                  60
ttt cat cag cgg tcc ggc aag gcc gac aag tac ctc aag tcg ctg ccg       240
Phe His Gln Arg Ser Gly Lys Ala Asp Lys Tyr Leu Lys Ser Leu Pro
65                  70                  75                  80
aag ctg gat gcg tcc aag gtg gag tcg cgg ttc tcg gcc aaa gag cag       288
Lys Leu Asp Ala Ser Lys Val Glu Ser Arg Phe Ser Ala Lys Glu Gln
                85                  90                  95
gcg cgg cgc gac gcc atg acg cgc gac tac gcg gcc ttt cgc gag gag       336
Ala Arg Arg Asp Ala Met Thr Arg Asp Tyr Ala Ala Phe Arg Glu Glu
            100                 105                 110
ctc gtc gcc gag ggg tac ttt gac ccg tcg atc ccg cac atg att tac       384
Leu Val Ala Glu Gly Tyr Phe Asp Pro Ser Ile Pro His Met Ile Tyr
        115                 120                 125
cgc gtc gtg gag atc gtg gcg ctc ttc gcg ctc tcg ttc tgg ctc atg       432
Arg Val Val Glu Ile Val Ala Leu Phe Ala Leu Ser Phe Trp Leu Met
    130                 135                 140
tcc aag gcc tcg ccc acc tcg ctc gtg ctg ggc gtg gtg atg aac ggc       480
Ser Lys Ala Ser Pro Thr Ser Leu Val Leu Gly Val Val Met Asn Gly
145                 150                 155                 160
att gcg cag ggc cgc tgc ggc tgg gtc atg cac gag atg ggc cac ggg       528
Ile Ala Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly
                165                 170                 175
tcg ttc acg ggc gtc atc tgg ctc gac gac cgg atg tgc gag ttc ttc       576
Ser Phe Thr Gly Val Ile Trp Leu Asp Asp Arg Met Cys Glu Phe Phe
            180                 185                 190
tac ggc gtc ggc tgc ggc atg agc ggg cac tac tgg aag aac cag cac       624
Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln His
        195                 200                 205
agc aag cac cac gcc gcg ccc aac cgc ctc gag cac gat gtc gat ctc       672
Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val Asp Leu
    210                 215                 220
aac acg ctg ccc ctg gtc gcc ttt aac gag cgc gtc gtg cgc aag gtc       720
Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val Arg Lys Val
225                 230                 235                 240
aag ccg gga tcg ctg ctg gcg ctc tgg ctg cgc gtg cag gcg tac ctc       768
Lys Pro Gly Ser Leu Leu Ala Leu Trp Leu Arg Val Gln Ala Tyr Leu
                245                 250                 255
ttt gcg ccc gtc tcg tgc ctg ctc atc ggc ctt ggc tgg acg ctc tac       816
Phe Ala Pro Val Ser Cys Leu Leu Ile Gly Leu Gly Trp Thr Leu Tyr
            260                 265                 270
ctg cac ccg cgc tac atg ctg cgc acc aag cgg cac atg gag ttc gtc       864
Leu His Pro Arg Tyr Met Leu Arg Thr Lys Arg His Met Glu Phe Val
        275                 280                 285
tgg atc ttc gcg cgc tac att ggc tgg ttc tcg ctc atg ggc gct ctc       912
Trp Ile Phe Ala Arg Tyr Ile Gly Trp Phe Ser Leu Met Gly Ala Leu
    290                 295                 300
ggc tac tcg ccg ggc acc tcg gtc ggg atg tac ctg tgc tcg ttc ggc       960
Gly Tyr Ser Pro Gly Thr Ser Val Gly Met Tyr Leu Cys Ser Phe Gly
305                 310                 315                 320
ctc ggc tgc att tac att ttc ctg cag ttc gcc gtc agc cac acg cac      1008
Leu Gly Cys Ile Tyr Ile Phe Leu Gln Phe Ala Val Ser His Thr His
                325                 330                 335
ctg ccg gtg acc aac ccg gag gac cag ctg cac tgg ctc gag tac gcg      1056
Leu Pro Val Thr Asn Pro Glu Asp Gln Leu His Trp Leu Glu Tyr Ala
            340                 345                 350
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gcc gac cac acg gtg aac att agc acc aag tcc tgg ctc gtc acg tgg        1104
Ala Asp His Thr Val Asn Ile Ser Thr Lys Ser Trp Leu Val Thr Trp
        355                 360                 365
tgg atg tcg aac ctg aac ttt cag atc gag cac cac ctc ttc ccc acg        1152
Trp Met Ser Asn Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
    370                 375                 380
gcg ccg cag ttc cgc ttc aag gaa atc agt cct cgc gtc gag gcc ctc        1200
Ala Pro Gln Phe Arg Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu
385                 390                 395                 400
ttc aag cgc cac aac ctc ccg tac tac gac ctg ccc tac acg agc gcg        1248
Phe Lys Arg His Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Ala
                405                 410                 415
gtc tcg acc acc ttt gcc aat ctt tat tcc gtc ggc cac tcg gtc ggc        1296
Val Ser Thr Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly
            420                 425                 430
gcc gac acc aag aag cag gac tga                                       1320
Ala Asp Thr Lys Lys Gln Asp
        435                 440
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<210> 52
<211> 439
<212> PRT
<213> Thraustochytrium ssp.

<400> 52

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Met Gly Lys Gly Ser Glu Gly Arg Ser Ala Ala Arg Glu Met Thr Ala
1               5                  10                  15
Glu Ala Asn Gly Asp Lys Arg Lys Thr Ile Leu Ile Glu Gly Val Leu
            20                  25                  30
Tyr Asp Ala Thr Asn Phe Lys His Pro Gly Gly Ser Ile Ile Asn Phe
        35                  40                  45
Leu Thr Glu Gly Glu Ala Gly Val Asp Ala Thr Gln Ala Tyr Arg Glu
    50                  55                  60
Phe His Gln Arg Ser Gly Lys Ala Asp Lys Tyr Leu Lys Ser Leu Pro
65                  70                  75                  80
Lys Leu Asp Ala Ser Lys Val Glu Ser Arg Phe Ser Ala Lys Glu Gln
                85                  90                  95
Ala Arg Arg Asp Ala Met Thr Arg Asp Tyr Ala Ala Phe Arg Glu Glu
            100                 105                 110
Leu Val Ala Glu Gly Tyr Phe Asp Pro Ser Ile Pro His Met Ile Tyr
        115                 120                 125
Arg Val Val Glu Ile Val Ala Leu Phe Ala Leu Ser Phe Trp Leu Met
    130                 135                 140
Ser Lys Ala Ser Pro Thr Ser Leu Val Leu Gly Val Val Met Asn Gly
145                 150                 155                 160
Ile Ala Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly
                165                 170                 175
Ser Phe Thr Gly Val Ile Trp Leu Asp Asp Arg Met Cys Glu Phe Phe
            180                 185                 190
Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln His
        195                 200                 205
Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val Asp Leu
    210                 215                 220
Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val Arg Lys Val
225                 230                 235                 240
Lys Pro Gly Ser Leu Leu Ala Leu Trp Leu Arg Val Gln Ala Tyr Leu
                245                 250                 255
Phe Ala Pro Val Ser Cys Leu Leu Ile Gly Leu Gly Trp Thr Leu Tyr
            260                 265                 270
Leu His Pro Arg Tyr Met Leu Arg Thr Lys Arg His Met Glu Phe Val
        275                 280                 285
Trp Ile Phe Ala Arg Tyr Ile Gly Trp Phe Ser Leu Met Gly Ala Leu
    290                 295                 300
Gly Tyr Ser Pro Gly Thr Ser Val Gly Met Tyr Leu Cys Ser Phe Gly
305                 310                 315                 320
Leu Gly Cys Ile Tyr Ile Phe Leu Gln Phe Ala Val Ser His Thr His
                325                 330                 335
Leu Pro Val Thr Asn Pro Glu Asp Gln Leu His Trp Leu Glu Tyr Ala
```

```
                340                 345                 350
Ala Asp His Thr Val Asn Ile Ser Thr Lys Ser Trp Leu Val Thr Trp
            355                 360                 365
Trp Met Ser Asn Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
        370                 375                 380
Ala Pro Gln Phe Arg Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu
385                 390                 395                 400
Phe Lys Arg His Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Ala
                405                 410                 415
Val Ser Thr Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly
            420                 425                 430
Ala Asp Thr Lys Lys Gln Asp
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gcg ttc gac ggt gag cgc gag cgg gcg gag gca aac gtg aag ctg tcc        96
Ala Phe Asp Gly Glu Arg Glu Arg Ala Glu Ala Asn Val Lys Leu Ser
            20                  25                  30
gcg gag aag atg gag ccg gcg gcg ctg gcg aag acg ttc gcg agg cgg       144
Ala Glu Lys Met Glu Pro Ala Ala Leu Ala Lys Thr Phe Ala Arg Arg
        35                  40                  45
tac gtc gtg atc gag ggg gtg gag tac gat gtg acg gat ttt aag cac       192
Tyr Val Val Ile Glu Gly Val Glu Tyr Asp Val Thr Asp Phe Lys His
    50                  55                  60
ccg gga gga acg gtt att ttc tat gcg ttg tca aac acc ggg gcg gac       240
Pro Gly Gly Thr Val Ile Phe Tyr Ala Leu Ser Asn Thr Gly Ala Asp
65                  70                  75                  80
gcg acg gaa gcg ttc aag gag ttt cat cat cgg tcg aga aag gcg agg       288
Ala Thr Glu Ala Phe Lys Glu Phe His His Arg Ser Arg Lys Ala Arg
                85                  90                  95
aaa gcc ttg gcg gcg ctc ccg tct cga ccg gcc aag acg gcc aag gtg       336
Lys Ala Leu Ala Ala Leu Pro Ser Arg Pro Ala Lys Thr Ala Lys Val
            100                 105                 110
gac gac gcg gag atg ctc caa gat ttc gcc aag tgg cgg aaa gaa ttg       384
Asp Asp Ala Glu Met Leu Gln Asp Phe Ala Lys Trp Arg Lys Glu Leu
        115                 120                 125
gag aga gat gga ttc ttc aag ccc tct ccg gcg cac gtg gcg tat cgc       432
Glu Arg Asp Gly Phe Phe Lys Pro Ser Pro Ala His Val Ala Tyr Arg
    130                 135                 140
ttc gcc gag ctc gcg gcg atg tac gct ctc ggg acg tac ctg atg tac       480
Phe Ala Glu Leu Ala Ala Met Tyr Ala Leu Gly Thr Tyr Leu Met Tyr
145                 150                 155                 160
gct cga tac gtc gtc tcc tcg gtg ctc gtg tac gct tgc ttt ttc ggc       528
Ala Arg Tyr Val Val Ser Ser Val Leu Val Tyr Ala Cys Phe Phe Gly
                165                 170                 175
gcc cga tgc ggt tgg gtg cag cac gag ggc gga cac agc tcg ctg acg       576
Ala Arg Cys Gly Trp Val Gln His Glu Gly Gly His Ser Ser Leu Thr
            180                 185                 190
ggc aac att tgg tgg gac aag cgc atc cag gcc ttc aca gcc ggg ttc       624
Gly Asn Ile Trp Trp Asp Lys Arg Ile Gln Ala Phe Thr Ala Gly Phe
        195                 200                 205
ggt ctc gcc ggt agc ggc gac atg tgg aac tcg atg cac aac aag cat       672
Gly Leu Ala Gly Ser Gly Asp Met Trp Asn Ser Met His Asn Lys His
    210                 215                 220
cac gcg acg cct caa aag gtt cgt cac gac atg gat ctg gac acc acc       720
His Ala Thr Pro Gln Lys Val Arg His Asp Met Asp Leu Asp Thr Thr
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225                 230                 235                 240
ccc gcg gtg gcg ttc ttc aac acc gcg gtg gaa gac aat cgt ccc cgt        768
Pro Ala Val Ala Phe Phe Asn Thr Ala Val Glu Asp Asn Arg Pro Arg
                245                 250                 255
ggc ttt agc aag tac tgg ttg cgc ctt cag gcg tgg acc ttc atc ccc        816
Gly Phe Ser Lys Tyr Trp Leu Arg Leu Gln Ala Trp Thr Phe Ile Pro
            260                 265                 270
gtg acg tcc ggc ttg gtg ctc ctt ttc tgg atg ttt ttc ctc cac ccc        864
Val Thr Ser Gly Leu Val Leu Leu Phe Trp Met Phe Phe Leu His Pro
        275                 280                 285
tcc aag gct ttg aag ggt ggc aag tac gaa gag ttg gtg tgg atg ctc        912
Ser Lys Ala Leu Lys Gly Gly Lys Tyr Glu Glu Leu Val Trp Met Leu
    290                 295                 300
gcc gcg cac gtc atc cgc acg tgg acg atc aag gcg gtg acc gga ttc        960
Ala Ala His Val Ile Arg Thr Trp Thr Ile Lys Ala Val Thr Gly Phe
305                 310                 315                 320
acc gcg atg cag tcc tac ggc tta ttt ttg gcg acg agc tgg gtg agc       1008
Thr Ala Met Gln Ser Tyr Gly Leu Phe Leu Ala Thr Ser Trp Val Ser
                325                 330                 335
ggc tgc tat ctg ttt gca cac ttc tcc acg tcg cac acg cac ctg gat       1056
Gly Cys Tyr Leu Phe Ala His Phe Ser Thr Ser His Thr His Leu Asp
            340                 345                 350
gtg gtg ccc gcg gac gag cat ctc tcc tgg gtt cga tac gcc gtc gat       1104
Val Val Pro Ala Asp Glu His Leu Ser Trp Val Arg Tyr Ala Val Asp
        355                 360                 365
cac acg atc gac atc gat ccg agt caa ggt tgg gtg aac tgg ttg atg       1152
His Thr Ile Asp Ile Asp Pro Ser Gln Gly Trp Val Asn Trp Leu Met
    370                 375                 380
ggc tac ctc aac tgc caa gtc atc cac cac ctc ttt ccg agc atg ccg       1200
Gly Tyr Leu Asn Cys Gln Val Ile His His Leu Phe Pro Ser Met Pro
385                 390                 395                 400
cag ttc cgc cag ccc gag gta tct cgc cgc ttc gtc gcc ttt gcg aaa       1248
Gln Phe Arg Gln Pro Glu Val Ser Arg Arg Phe Val Ala Phe Ala Lys
                405                 410                 415
aag tgg aac ctc aac tac aag gtc atg acc tac gcc ggt gcg tgg aag       1296
Lys Trp Asn Leu Asn Tyr Lys Val Met Thr Tyr Ala Gly Ala Trp Lys
            420                 425                 430
gca acg ctc gga aac ctc gac aac gtg ggt aag cac tac tac gtg cac       1344
Ala Thr Leu Gly Asn Leu Asp Asn Val Gly Lys His Tyr Tyr Val His
        435                 440                 445
ggc caa cac tcc gga aag acg gcg taa                                  1371
Gly Gln His Ser Gly Lys Thr Ala
    450                 455
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<213> Ostreococcus tauri

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            20                  25                  30
Ala Glu Lys Met Glu Pro Ala Ala Leu Ala Lys Thr Phe Ala Arg Arg
        35                  40                  45
Tyr Val Val Ile Glu Gly Val Glu Tyr Asp Val Thr Asp Phe Lys His
    50                  55                  60
Pro Gly Gly Thr Val Ile Phe Tyr Ala Leu Ser Asn Thr Gly Ala Asp
65                  70                  75                  80
Ala Thr Glu Ala Phe Lys Glu Phe His His Arg Ser Arg Lys Ala Arg
                85                  90                  95
Lys Ala Leu Ala Ala Leu Pro Ser Arg Pro Ala Lys Thr Ala Lys Val
            100                 105                 110
Asp Asp Ala Glu Met Leu Gln Asp Phe Ala Lys Trp Arg Lys Glu Leu
        115                 120                 125
Glu Arg Asp Gly Phe Phe Lys Pro Ser Pro Ala His Val Ala Tyr Arg
    130                 135                 140
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Phe Ala Glu Leu Ala Ala Met Tyr Ala Leu Gly Thr Tyr Leu Met Tyr
145                 150                 155                 160
Ala Arg Tyr Val Val Ser Ser Val Leu Val Tyr Ala Cys Phe Phe Gly
                165                 170                 175
Ala Arg Cys Gly Trp Val Gln His Glu Gly Gly His Ser Ser Leu Thr
            180                 185                 190
Gly Asn Ile Trp Trp Asp Lys Arg Ile Gln Ala Phe Thr Ala Gly Phe
        195                 200                 205
Gly Leu Ala Gly Ser Gly Asp Met Trp Asn Ser Met His Asn Lys His
    210                 215                 220
His Ala Thr Pro Gln Lys Val Arg His Asp Met Asp Leu Asp Thr Thr
225                 230                 235                 240
Pro Ala Val Ala Phe Phe Asn Thr Ala Val Glu Asp Asn Arg Pro Arg
                245                 250                 255
Gly Phe Ser Lys Tyr Trp Leu Arg Leu Gln Ala Trp Thr Phe Ile Pro
            260                 265                 270
Val Thr Ser Gly Leu Val Leu Leu Phe Trp Met Phe Phe Leu His Pro
        275                 280                 285
Ser Lys Ala Leu Lys Gly Gly Lys Tyr Glu Glu Leu Val Trp Met Leu
    290                 295                 300
Ala Ala His Val Ile Arg Thr Trp Thr Ile Lys Ala Val Thr Gly Phe
305                 310                 315                 320
Thr Ala Met Gln Ser Tyr Gly Leu Phe Leu Ala Thr Ser Trp Val Ser
                325                 330                 335
Gly Cys Tyr Leu Phe Ala His Phe Ser Thr Ser His Thr His Leu Asp
            340                 345                 350
Val Val Pro Ala Asp Glu His Leu Ser Trp Val Arg Tyr Ala Val Asp
        355                 360                 365
His Thr Ile Asp Ile Asp Pro Ser Gln Gly Trp Val Asn Trp Leu Met
    370                 375                 380
Gly Tyr Leu Asn Cys Gln Val Ile His His Leu Phe Pro Ser Met Pro
385                 390                 395                 400
Gln Phe Arg Gln Pro Glu Val Ser Arg Arg Phe Val Ala Phe Ala Lys
                405                 410                 415
Lys Trp Asn Leu Asn Tyr Lys Val Met Thr Tyr Ala Gly Ala Trp Lys
            420                 425                 430
Ala Thr Leu Gly Asn Leu Asp Asn Val Gly Lys His Tyr Tyr Val His
        435                 440                 445
Gly Gln His Ser Gly Lys Thr Ala
    450                 455

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1               5                   10                  15
gat ggc gtc ctt tac gac tgc acc gat ttc cgg cat ccg ggt ggc agc        96
Asp Gly Val Leu Tyr Asp Cys Thr Asp Phe Arg His Pro Gly Gly Ser
            20                  25                  30
att ctg aaa tac tac ctc ggc agc ggc gac gcc acc gag acg tac caa       144
Ile Leu Lys Tyr Tyr Leu Gly Ser Gly Asp Ala Thr Glu Thr Tyr Gln
        35                  40                  45
cag ttc cac ttg aag ctg ccc agg gcg gac aag tat ctc aag cgg ctg       192
Gln Phe His Leu Lys Leu Pro Arg Ala Asp Lys Tyr Leu Lys Arg Leu
    50                  55                  60
ccg aat cgc ccg gcg ccg cca cag cac agc gtc aac gtg gat gag cag       240
Pro Asn Arg Pro Ala Pro Pro Gln His Ser Val Asn Val Asp Glu Gln
65                  70                  75                  80
aag cga ttg gag aag ctc tcg cgg gac ttc aag gcg ctg cag gat gcg       288
Lys Arg Leu Glu Lys Leu Ser Arg Asp Phe Lys Ala Leu Gln Asp Ala
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                    85                  90                  95
tgc gta gag gag ggc ctt ttt aac gcc agc tgg ccg cac atc gtc tac        336
Cys Val Glu Glu Gly Leu Phe Asn Ala Ser Trp Pro His Ile Val Tyr
            100                 105                 110
cgg ttt tct gag ctg atc ctg atg cac gcc atc ggt ctt tac atg ctc        384
Arg Phe Ser Glu Leu Ile Leu Met His Ala Ile Gly Leu Tyr Met Leu
        115                 120                 125
ttc cgt ctt ccg atc ctg tgg ccc gtc gcg ctg gtg atc ctt gga gtg        432
Phe Arg Leu Pro Ile Leu Trp Pro Val Ala Leu Val Ile Leu Gly Val
    130                 135                 140
gcg gag ggg cga tgt ggc tgg tgg atg cac gag gcc ggt cac tac agc        480
Ala Glu Gly Arg Cys Gly Trp Trp Met His Glu Ala Gly His Tyr Ser
145                 150                 155                 160
gtc aca ggc att ccg tgg ttg gac att aaa ata cag gag gta ctc tac        528
Val Thr Gly Ile Pro Trp Leu Asp Ile Lys Ile Gln Glu Val Leu Tyr
                165                 170                 175
gga ctt ggc gat gga atg agc gcg tcg tgg tgg cgg tcg cag cat aac        576
Gly Leu Gly Asp Gly Met Ser Ala Ser Trp Trp Arg Ser Gln His Asn
            180                 185                 190
aag cat cac gct act ccg cag aag cac cgg cac gac gtg gac ctt gag        624
Lys His His Ala Thr Pro Gln Lys His Arg His Asp Val Asp Leu Glu
        195                 200                 205
acg ctg cct ctc gtc gcc ttc aac aag atc atc gcg cgc cgc ggc aag        672
Thr Leu Pro Leu Val Ala Phe Asn Lys Ile Ile Ala Arg Arg Gly Lys
    210                 215                 220
agg aac gcg agc att cgc cgc tgg atc tcc ctg cag atg ttc ctc ttc        720
Arg Asn Ala Ser Ile Arg Arg Trp Ile Ser Leu Gln Met Phe Leu Phe
225                 230                 235                 240
ggc ccc gtc acc tgc tcc ctt gtc gcc ctc tac tgg cag ctc ttc cta        768
Gly Pro Val Thr Cys Ser Leu Val Ala Leu Tyr Trp Gln Leu Phe Leu
                245                 250                 255
cac gtc cgc cac gcc atg cgc act cag cgt tac aca gag ggc tct gcc        816
His Val Arg His Ala Met Arg Thr Gln Arg Tyr Thr Glu Gly Ser Ala
            260                 265                 270
atc ctg tgc cgc tgg atc gtg gtc ggc gtt atc tgt cac cag ctg cag        864
Ile Leu Cys Arg Trp Ile Val Val Gly Val Ile Cys His Gln Leu Gln
        275                 280                 285
gtc tcg ttc tgg caa ggc ctc ggc ggc gtt ctc ttc tcc cag gcc ttc        912
Val Ser Phe Trp Gln Gly Leu Gly Gly Val Leu Phe Ser Gln Ala Phe
    290                 295                 300
agc gcc gcc tac atc ttc ata aac ttc gcc ctc aac cac tct cac ctg        960
Ser Ala Ala Tyr Ile Phe Ile Asn Phe Ala Leu Asn His Ser His Leu
305                 310                 315                 320
ccg atg ctt ccg gaa gac gaa cac gcg cac ttc gtc gag tac gcg gcc       1008
Pro Met Leu Pro Glu Asp Glu His Ala His Phe Val Glu Tyr Ala Ala
                325                 330                 335
atc tac acc atg aac gtg aca ccg tcg tgg ttc gtg acg tgg ttc atg       1056
Ile Tyr Thr Met Asn Val Thr Pro Ser Trp Phe Val Thr Trp Phe Met
            340                 345                 350
ggc tac ctt aac tac cag gtg gaa cac cac ctc ttc cct acc atg cca       1104
Gly Tyr Leu Asn Tyr Gln Val Glu His His Leu Phe Pro Thr Met Pro
        355                 360                 365
cag ttc cgc ttc gtc caa ctg gcg ccg cga gtg cgg aaa ctt ttt gag       1152
Gln Phe Arg Phe Val Gln Leu Ala Pro Arg Val Arg Lys Leu Phe Glu
    370                 375                 380
gaa aac ggc ctc aag tac gat tcg cgt ccg tac atg gag tcg ctc cag       1200
Glu Asn Gly Leu Lys Tyr Asp Ser Arg Pro Tyr Met Glu Ser Leu Gln
385                 390                 395                 400
aaa acc ttc aag aac ctc ggc gac gtg gcc gag ttc atc gtt gct ggg       1248
Lys Thr Phe Lys Asn Leu Gly Asp Val Ala Glu Phe Ile Val Ala Gly
                405                 410                 415
aac taa                                                             1254
Asn
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<213> Leishmania major

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            20                  25                  30
Ile Leu Lys Tyr Tyr Leu Gly Ser Gly Asp Ala Thr Glu Thr Tyr Gln
        35                  40                  45
Gln Phe His Leu Lys Leu Pro Arg Ala Asp Lys Tyr Leu Lys Arg Leu
    50                  55                  60
Pro Asn Arg Pro Ala Pro Pro Gln His Ser Val Asn Val Asp Glu Gln
65                  70                  75                  80
Lys Arg Leu Glu Lys Leu Ser Arg Asp Phe Lys Ala Leu Gln Asp Ala
                85                  90                  95
Cys Val Glu Glu Gly Leu Phe Asn Ala Ser Trp Pro His Ile Val Tyr
            100                 105                 110
Arg Phe Ser Glu Leu Ile Leu Met His Ala Ile Gly Leu Tyr Met Leu
        115                 120                 125
Phe Arg Leu Pro Ile Leu Trp Pro Val Ala Leu Val Ile Leu Gly Val
    130                 135                 140
Ala Glu Gly Arg Cys Gly Trp Trp Met His Glu Ala Gly His Tyr Ser
145                 150                 155                 160
Val Thr Gly Ile Pro Trp Leu Asp Ile Lys Ile Gln Glu Val Leu Tyr
                165                 170                 175
Gly Leu Gly Asp Gly Met Ser Ala Ser Trp Trp Arg Ser Gln His Asn
            180                 185                 190
Lys His His Ala Thr Pro Gln Lys His Arg His Asp Val Asp Leu Glu
        195                 200                 205
Thr Leu Pro Leu Val Ala Phe Asn Lys Ile Ile Ala Arg Arg Gly Lys
    210                 215                 220
Arg Asn Ala Ser Ile Arg Arg Trp Ile Ser Leu Gln Met Phe Leu Phe
225                 230                 235                 240
Gly Pro Val Thr Cys Ser Leu Val Ala Leu Tyr Trp Gln Leu Phe Leu
                245                 250                 255
His Val Arg His Ala Met Arg Thr Gln Arg Tyr Thr Glu Gly Ser Ala
            260                 265                 270
Ile Leu Cys Arg Trp Ile Val Val Gly Val Ile Cys His Gln Leu Gln
        275                 280                 285
Val Ser Phe Trp Gln Gly Leu Gly Gly Val Leu Phe Ser Gln Ala Phe
    290                 295                 300
Ser Ala Ala Tyr Ile Phe Ile Asn Phe Ala Leu Asn His Ser His Leu
305                 310                 315                 320
Pro Met Leu Pro Glu Asp Glu His Ala His Phe Val Glu Tyr Ala Ala
                325                 330                 335
Ile Tyr Thr Met Asn Val Thr Pro Ser Trp Phe Val Thr Trp Phe Met
            340                 345                 350
Gly Tyr Leu Asn Tyr Gln Val Glu His His Leu Phe Pro Thr Met Pro
        355                 360                 365
Gln Phe Arg Phe Val Gln Leu Ala Pro Arg Val Arg Lys Leu Phe Glu
    370                 375                 380
Glu Asn Gly Leu Lys Tyr Asp Ser Arg Pro Tyr Met Glu Ser Leu Gln
385                 390                 395                 400
Lys Thr Phe Lys Asn Leu Gly Asp Val Ala Glu Phe Ile Val Ala Gly
                405                 410                 415
Asn
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<220>
<221> Variant
<222> (345)..(346)
<223> xaa in position 345 to 346 is any amino acid
<220>
<221> Variant
<222> (349)..(349)
<223> xaa in position 349 is any amino acid
<220>
<221> Variant
<222> (351)..(352)
<223> xaa in position 351 to 352 is any amino acid

<220>
<221> Variant
<222> (358)..(359)
<223> xaa in position 358 to 359 is any amino acid
<220>
<221> Variant
<222> (364)..(369)
<223> xaa in position 364 to 369 is any amino acid
<220>
<221> Variant
<222> (371)..(379)
<223> xaa in position 371 to 379 is any amino acid
<220>
<221> Variant
<222> (381)..(381)
<223> xaa in position 381 is any amino acid
<220>
<221> Variant
<222> (383)..(386)
<223> xaa in position 383 to 386 is any amino acid
<220>
<221> Variant
<222> (388)..(393)
<223> xaa in position 388 to 393 is any amino acid
<220>
<221> Variant
<222> (395)..(396)
<223> xaa in position 395 to 396 is any amino acid
<220>
<221> Variant
<222> (399)..(400)
<223> xaa in position 399 to 400 is any amino acid

<400> 57
Ile xaa Gly Val xaa Tyr Asp xaa Thr xaa Phe xaa His Pro Gly Gly
1 5 10 15
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Asp Ala Thr
20 25 30
xaa xaa xaa xaa xaa Phe His xaa xaa xaa xaa xaa Ala xaa Lys xaa
35 40 45
Leu xaa xaa Leu Pro xaa xaa xaa Ala xaa xaa xaa xaa xaa xaa xaa
50 55 60
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Asp xaa xaa
65 70 75 80
xaa xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa Phe xaa xaa Ser xaa
85 90 95
xaa His xaa xaa Tyr Arg xaa xaa Glu xaa xaa xaa xaa xaa Ala xaa
100 105 110
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
115 120 125
xaa Val xaa xaa xaa xaa xaa xaa xaa Arg Cys Gly Trp xaa xaa His
130 135 140
Glu xaa Gly His xaa Ser xaa Thr Gly xaa xaa Trp xaa Asp xaa xaa
145 150 155 160
xaa xaa xaa xaa xaa xaa Gly xaa Gly xaa xaa xaa Ser xaa xaa xaa
165 170 175
Trp xaa xaa xaa His xaa Lys His His Ala xaa Pro xaa xaa xaa xaa
180 185 190
His Asp xaa Asp Leu xaa Thr xaa Pro xaa Val Ala Phe xaa xaa xaa
195 200 205
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Trp xaa xaa
210 215 220
xaa Gln xaa xaa xaa Phe xaa Pro Val xaa xaa xaa Leu xaa xaa Leu
225 230 235 240
xaa Trp xaa xaa xaa Leu His xaa xaa xaa xaa xaa xaa xaa xaa xaa
245 250 255
xaa xaa Glu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
260 265 270

```
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa
        275                 280                 285
xaa Leu xaa xaa xaa xaa xaa xaa xaa xaa Tyr xaa Phe xaa xaa Phe
    290                 295                 300
xaa xaa xaa His xaa His Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa
305                 310                 315                 320
xaa xaa xaa xaa Tyr Ala xaa xaa xaa Thr xaa xaa xaa xaa xaa xaa
                325                 330                 335
xaa xaa xaa Val xaa Trp xaa Met xaa xaa Leu Asn xaa Gln xaa xaa
            340                 345                 350
His His Leu Phe Pro xaa xaa Pro Gln Phe Arg xaa xaa xaa xaa xaa
        355                 360                 365
xaa Arg xaa xaa xaa xaa xaa xaa xaa xaa xaa Leu xaa Tyr xaa xaa
    370                 375                 380
xaa xaa Tyr xaa xaa xaa xaa xaa xaa Thr xaa xaa Asn Leu xaa xaa
385                 390                 395                 400
Val
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<210> 58
<211> 59
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (3)..(4)
<223> xaa in position 3 to 4 is any amino acid
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is any amino acid
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Phe, Leu or Val
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is any or no amino acid
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<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any or no amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is any amino acid
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Lys or Arg
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Ile or Met
<220>
<221> Variant
<222> (24)..(24)

<223> xaa in position 24 is any amino acid
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is Ala or Glu
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is Phe or Val
<220>
<221> Variant
<222> (27)..(28)
<223> xaa in position 27 to 28 is any amino acid
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Phe, Leu or Val
<220>
<221> Variant
<222> (32)..(32)
<223> xaa in position 32 is any amino acid
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Ala or Gly
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is any amino acid
<220>
<221> Variant
<222> (36)..(36)
<223> xaa in position 36 is Ala or Gly
<220>
<221> Variant
<222> (37)..(38)
<223> xaa in position 37 to 38 is any amino acid
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is any amino acid
<220>
<221> Variant
<222> (41)..(41)
<223> xaa in position 41 is Asn or Ser
<220>
<221> Variant
<222> (42)..(42)
<223> xaa in position 42 is any amino acid
<220>
<221> Variant
<222> (44)..(44)
<223> xaa in position 44 is Asn or Ser
<220>
<221> Variant
<222> (49)..(49)
<223> xaa in position 49 is Ala or Thr
<220>
<221> Variant
<222> (51)..(51)
<223> xaa in position 51 is Asn or Gln
<220>
<221> Variant
<222> (52)..(52)
<223> xaa in position 52 is Lys or Arg
<220>
<221> Variant
<222> (53)..(53)

<223> xaa in position 53 is any amino acid
<220>
<221> Variant
<222> (54)..(54)
<223> xaa in position 54 is Glu or Arg
<220>
<221> Variant
<222> (57)..(57)
<223> xaa in position 57 is Met or Val

<400> 58
Gly Trp xaa xaa His Glu xaa Gly His xaa Ser xaa Thr Gly xaa Ile
1 5 10 15
xaa Trp xaa Asp xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa Gly xaa
20 25 30
xaa xaa Ser xaa xaa xaa Trp xaa xaa xaa His xaa Lys His His Ala
35 40 45
xaa Pro xaa xaa xaa xaa His Asp xaa Asp Leu
50 55

<210> 59
<211> 50
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Asn or Thr
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is any amino acid
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any amino acid
<220>
<221> Variant
<222> (7)..(8)
<223> xaa in position 7 to 8 is any or no amino acid
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (11)..(12)
<223> xaa in position 11 to 12 is any or no amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Ile or Val
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ser or Thr
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is any amino acid
<220>
<221> Variant

<222> (27)..(28)
<223> xaa in position 27 to 28 is any amino acid
<220>
<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is Glu or Gln
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Ile, Leu or Val
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is Ala or Ser
<220>
<221> Variant
<222> (32)..(32)
<223> xaa in position 32 is any amino acid
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Phe or Val
<220>
<221> Variant
<222> (35)..(36)
<223> xaa in position 35 to 36 is any amino acid
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Phe or Leu
<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is any amino acid
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Glu or Lys
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is Glu, Lys or Arg
<220>
<221> Variant
<222> (41)..(41)
<223> xaa in position 41 is any amino acid
<220>
<221> Variant
<222> (42)..(42)
<223> xaa in position 42 is Gly or Asn
<220>
<221> Variant
<222> (44)..(44)
<223> xaa in position 44 is any amino acid
<220>
<221> Variant
<222> (46)..(46)
<223> xaa in position 46 is any amino acid
<220>
<221> Variant
<222> (47)..(47)
<223> xaa in position 47 is Asp, Ser or Val
<220>
<221> Variant
<222> (48)..(48)
<223> xaa in position 48 is any amino acid
<220>
<221> Variant

<222> (49)..(49)
<223> xaa in position 49 is Pro or Thr

<400> 59
Val xaa Trp xaa Met xaa xaa xaa Asn xaa xaa xaa Gln xaa xaa His
 1   5   10   15
His Leu Phe Pro xaa xaa Pro Gln Phe Arg xaa xaa xaa xaa xaa xaa
            20   25   30
Arg xaa xaa xaa xaa xaa xaa xaa xaa xaa Leu xaa Tyr xaa xaa xaa
        35   40   45
xaa Tyr
    50

<210> 60
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Asp or Glu
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is any amino acid
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Ala, Cys or Val
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is Asp or Asn
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Lys or Arg
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any or no amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Phe, Ile or Leu

<400> 60
Ile xaa Gly Val xaa Tyr Asp xaa Thr xaa Phe xaa His Pro Gly Gly
 1   5   10   15
xaa xaa Ile xaa
            20

<210> 61
<211> 61
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is any amino acid

<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Ala or Leu
<220>
<221> Variant
<222> (8)..(10)
<223> xaa in position 8 to 10 is any amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is Ala, Ile or Val
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Ala or Val
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Glu or Arg
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Asp, Lys or Arg
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Gly, Asn or Val
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Lys or Arg
<220>
<221> Variant
<222> (17)..(18)
<223> xaa in position 17 to 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Ala, Gly or Ser
<220>
<221> Variant
<222> (20)..(23)
<223> xaa in position 20 to 23 is any amino acid
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any or no amino acid
<220>
<221> Variant
<222> (28)..(29)
<223> xaa in position 28 to 29 is any or no amino acid
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is any amino acid
<220>
<221> Variant
<222> (32)..(32)
<223> xaa in position 32 is Phe, Trp or Tyr
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is any amino acid
<220>
<221> Variant
<222> (35)..(35)
<223> xaa in position 35 is Ala, Gly or Ile

<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is Ser or Thr
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Cys or Ser
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is any amino acid
<220>
<221> Variant
<222> (42)..(42)
<223> xaa in position 42 is Ile or Val
<220>
<221> Variant
<222> (43)..(43)
<223> xaa in position 43 is Ala, Gly or Leu
<220>
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<222> (45)..(45)
<223> xaa in position 45 is any amino acid
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<221> Variant
<222> (47)..(47)
<223> xaa in position 47 is any amino acid
<220>
<221> Variant
<222> (48)..(48)
<223> xaa in position 48 is Phe or Leu
<220>
<221> Variant
<222> (49)..(49)
<223> xaa in position 49 is Phe or Tyr
<220>
<221> Variant
<222> (52)..(52)
<223> xaa in position 52 is Pro or Val
<220>
<221> Variant
<222> (53)..(53)
<223> xaa in position 53 is Arg or Ser
<220>
<221> Variant
<222> (54)..(55)
<223> xaa in position 54 to 55 is any amino acid
<220>
<221> Variant
<222> (56)..(56)
<223> xaa in position 56 is Leu or Met
<220>
<221> Variant
<222> (57)..(57)
<223> xaa in position 57 is Lys or Arg
<220>
<221> Variant
<222> (58)..(58)
<223> xaa in position 58 is Gly or Thr
<220>
<221> Variant
<222> (59)..(59)
<223> xaa in position 59 is any amino acid
<220>
<221> Variant
<222> (60)..(60)
<223> xaa in position 60 is Lys or Arg

<220>
<221> Variant
<222> (61)..(61)
<223> xaa in position 61 is His or Tyr

<400> 61
Thr xaa Pro xaa Val Ala Phe xaa xaa xaa xaa xaa xaa xaa xaa xaa
  1   5   10   15
xaa xaa xaa xaa xaa xaa xaa Trp xaa xaa Leu xaa xaa Gln xaa xaa
            20   25   30
xaa Phe xaa Pro Val xaa xaa xaa Leu xaa xaa Leu xaa Trp xaa xaa
        35   40   45
xaa Leu His xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
    50   55   60

<210> 62
<211> 34
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(5)
<223> xaa in position 2 to 5 is any amino acid
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any or no amino acid
<220>
<221> Variant
<222> (8)..(9)
<223> xaa in position 8 to 9 is any or no amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is Asp or Pro
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Met or Val
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Asn or Pro
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is any amino acid
<220>
<221> Variant
<222> (16)..(17)
<223> xaa in position 16 to 17 is Asp or Glu
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Ala or Leu
<220>
<221> Variant
<222> (20)..(20)

<223> xaa in position 20 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Phe or Trp
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Leu or Val
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Glu or Arg
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is Ala or Val
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is any amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is His or Tyr
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Ile, Met or Val
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is Asp or Asn
<220>
<221> Variant
<222> (32)..(32)
<223> xaa in position 32 is Ile or Val
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Asp, Ser or Thr
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Pro or Thr

<400> 62
Ala xaa xaa xaa xaa xaa His xaa xaa Leu xaa xaa xaa xaa xaa xaa
  1   5   10   15
xaa xaa xaa xaa xaa xaa xaa Tyr Ala xaa xaa xaa Thr xaa xaa xaa
            20   25   30
xaa xaa

<210> 63
<211> 28
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Glu or Gln
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ala or Thr

<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Phe or Tyr
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is any amino acid
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Glu or Gln
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is any amino acid
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Lys or Arg
<220>
<221> Variant
<222> (13)..(14)
<223> xaa in position 13 to 14 is any amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Lys or Arg
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Asp or Arg
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is any amino acid
<220>
<221> Variant
<222> (21)..(22)
<223> xaa in position 21 to 22 is any amino acid
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Asp or Pro

<400> 63
Asp Ala Thr xaa xaa xaa xaa xaa Phe His xaa xaa xaa xaa xaa Ala
  1   5   10   15
xaa Lys xaa Leu xaa xaa Leu Pro xaa xaa xaa Ala
            20   25

<210> 64
<211> 903
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(903)

<400> 64
atg tct gct tct gga gct ttg ttg cct gct att gct ttc gct gct tac 48
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1   5   10   15

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gct tac gct acc tac gct tat gct ttc gag tgg tct cat gct aac gga          96
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
atc gat aac gtg gat gct aga gag tgg att gga gct ttg tct ttg aga         144
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
ctc cct gca att gct acc acc atg tac ctc ttg ttc tgc ctt gtg gga         192
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
    50                  55                  60
cct aga ttg atg gct aag agg gag gct ttt gat cct aag gga ttc atg         240
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
ctc gct tac aac gct tac caa acc gct ttc aac gtt gtg gtg ctc gga         288
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
atg ttc gct aga gag atc tct gga ttg gga caa cct gtt tgg gga tct         336
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
act atg cct tgg agc gat agg aag tcc ttc aag att ttg ttg gga gtg         384
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
tgg ctc cat tac aac aat aag tac ctc gag ttg ttg gat act gtg ttc         432
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
atg gtg gct agg aaa aag acc aag cag ctc tct ttc ttg cat gtg tac         480
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
cat cat gct ttg ttg att tgg gct tgg tgg ctt gtt tgt cat ctc atg         528
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
gct acc aac gat tgc atc gat gct tat ttc gga gct gct tgc aac tct         576
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
ttc atc cac atc gtg atg tac tcc tac tac ctc atg tct gct ttg gga         624
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
att aga tgc cct tgg aag aga tat atc acc cag gct cag atg ttg caa         672
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
    210                 215                 220
ttc gtg atc gtg ttc gct cat gct gtt ttc gtg ctc aga caa aag cac         720
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
tgc cct gtt act ttg cct tgg gca caa atg ttc gtg atg aca aat atg         768
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
ttg gtg ctc ttc gga aac ttc tac ctc aag gct tac tct aac aag tct         816
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
agg gga gat gga gct tct tct gtt aag cct gct gag act act aga gca         864
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
cct tct gtg aga aga acc agg tcc agg aag atc gat tga                     903
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300
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<210> 65
<211> 300
<212> PRT
<213> Ostreococcus tauri

<400> 65

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Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1               5                   10                  15
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
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    50                  55                  60
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
    210                 215                 220
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300

<210> 66
<211> 834
<212> DNA
<213> Pavlova sp

<220>
<221> CDS
<222> (1)..(834)

<400> 66
atg atg ttg gcc gca ggc tat ctt cta gtg ctc tcg gcc gct cgc cag      48
Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gln
1               5                   10                  15
agc ttc cag cag gac att gac aac ccc aac ggg gcc tac tcg acc tcg      96
Ser Phe Gln Gln Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
            20                  25                  30
tgg act ggc ctg ccc att gtg atg tct gtg gtc tat ctc agc ggt gtg     144
Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
        35                  40                  45
ttt ggg ctc aca aag tac ttc gag aac cgg aag ccc atg acg ggg ctg     192
Phe Gly Leu Thr Lys Tyr Phe Glu Asn Arg Lys Pro Met Thr Gly Leu
    50                  55                  60
aag gac tac atg ttc act tac aat ctc tac cag gtg atc atc aac gtg     240
Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gln Val Ile Ile Asn Val
65                  70                  75                  80
tgg tgc gtg gtg gcc ttt ctc ctg gag gtg cgg cgt gcg ggc atg tca     288
Trp Cys Val Val Ala Phe Leu Leu Glu Val Arg Arg Ala Gly Met Ser
                85                  90                  95
ctc atc ggc aat aag gtg gac ctt ggg ccc aac tcc ttc agg ctc ggc     336
Leu Ile Gly Asn Lys Val Asp Leu Gly Pro Asn Ser Phe Arg Leu Gly
            100                 105                 110
ttc gtc acg tgg gtg cac tac aac aac aag tac gtg gag ctc ctc gac     384
Phe Val Thr Trp Val His Tyr Asn Asn Lys Tyr Val Glu Leu Leu Asp
        115                 120                 125
acc cta tgg atg gtg ctg cgc aag aag acg cag cag gtc tcc ttc ctc     432
Thr Leu Trp Met Val Leu Arg Lys Lys Thr Gln Gln Val Ser Phe Leu
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    130                 135                 140
cac gtc tat cat cac gtg ctt ctg atg tgg gcc tgg ttc gtt gtc gtc        480
His Val Tyr His His Val Leu Leu Met Trp Ala Trp Phe Val Val Val
145                 150                 155                 160
aag ctc ggc aat ggt ggt gac gca tat ttt ggc ggt ctc atg aac tcg        528
Lys Leu Gly Asn Gly Gly Asp Ala Tyr Phe Gly Gly Leu Met Asn Ser
                165                 170                 175
atc atc cac gtg atg atg tat tcc tac tac acc atg gcg ctc ctg ggc        576
Ile Ile His Val Met Met Tyr Ser Tyr Tyr Thr Met Ala Leu Leu Gly
            180                 185                 190
tgg tca tgc ccc tgg aag cgc tac ctc acg cag gca cag ctc gtg cag        624
Trp Ser Cys Pro Trp Lys Arg Tyr Leu Thr Gln Ala Gln Leu Val Gln
        195                 200                 205
ttt tgc atc tgc ctc gcc cac tcc aca tgg gcg gca gta acg ggt gcc        672
Phe Cys Ile Cys Leu Ala His Ser Thr Trp Ala Ala Val Thr Gly Ala
    210                 215                 220
tac ccg tgg cga att tgc ttg gtg gag gtg tgg gtg atg gtg tcc atg        720
Tyr Pro Trp Arg Ile Cys Leu Val Glu Val Trp Val Met Val Ser Met
225                 230                 235                 240
ctg gtg ctc ttc aca cgc ttc tac cgc cag gcc tat gcc aag gag gcg        768
Leu Val Leu Phe Thr Arg Phe Tyr Arg Gln Ala Tyr Ala Lys Glu Ala
                245                 250                 255
aag gcc aag gag gcg aaa aag ctc gca cag gag gca tca cag gcc aag        816
Lys Ala Lys Glu Ala Lys Lys Leu Ala Gln Glu Ala Ser Gln Ala Lys
            260                 265                 270
gcg gtc aag gcg gag taa                                             834
Ala Val Lys Ala Glu
        275

<210> 67
<211> 277
<212> PRT
<213> Pavlova sp

<400> 67
Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gln
1                   5                   10                  15
Ser Phe Gln Gln Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
            20                  25                  30
Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
        35                  40                  45
Phe Gly Leu Thr Lys Tyr Phe Glu Asn Arg Lys Pro Met Thr Gly Leu
    50                  55                  60
Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gln Val Ile Ile Asn Val
65                  70                  75                  80
Trp Cys Val Val Ala Phe Leu Leu Glu Val Arg Arg Ala Gly Met Ser
                85                  90                  95
Leu Ile Gly Asn Lys Val Asp Leu Gly Pro Asn Ser Phe Arg Leu Gly
            100                 105                 110
Phe Val Thr Trp Val His Tyr Asn Asn Lys Tyr Val Glu Leu Leu Asp
        115                 120                 125
Thr Leu Trp Met Val Leu Arg Lys Lys Thr Gln Gln Val Ser Phe Leu
    130                 135                 140
His Val Tyr His His Val Leu Leu Met Trp Ala Trp Phe Val Val Val
145                 150                 155                 160
Lys Leu Gly Asn Gly Gly Asp Ala Tyr Phe Gly Gly Leu Met Asn Ser
                165                 170                 175
Ile Ile His Val Met Met Tyr Ser Tyr Tyr Thr Met Ala Leu Leu Gly
            180                 185                 190
Trp Ser Cys Pro Trp Lys Arg Tyr Leu Thr Gln Ala Gln Leu Val Gln
        195                 200                 205
Phe Cys Ile Cys Leu Ala His Ser Thr Trp Ala Ala Val Thr Gly Ala
    210                 215                 220
Tyr Pro Trp Arg Ile Cys Leu Val Glu Val Trp Val Met Val Ser Met
225                 230                 235                 240
Leu Val Leu Phe Thr Arg Phe Tyr Arg Gln Ala Tyr Ala Lys Glu Ala
                245                 250                 255
Lys Ala Lys Glu Ala Lys Lys Leu Ala Gln Glu Ala Ser Gln Ala Lys
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            260                 265                 270
Ala Val Lys Ala Glu
        275

<210> 68
<211> 1077
<212> DNA
<213> Thalassiosira pseudonana

<220>
<221> CDS
<222> (1)..(1077)

<400> 68
atg tgc tca tca ccg ccg tca caa tcc aaa aca aca tcc ctc cta gca        48
Met Cys Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala
1               5                  10                  15
cgg tac acc acc gcc gcc ctc ctc ctc ctc acc ctc aca aca tgg tgc        96
Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Trp Cys
            20                  25                  30
cac ttc gcc ttc cca gcc gcc acc gcc aca ccc ggc ctc acc gcc gaa       144
His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu
        35                  40                  45
atg cac tcc tac aaa gtc cca ctc ggt ctc acc gta ttc tac ctg ctg       192
Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu
    50                  55                  60
agt cta ccg tca cta aag tac gtt acg gac aac tac ctt gcc aaa aag       240
Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys
65                  70                  75                  80
tat gat atg aag tca ctc cta acg gaa tca atg gtg ttg tac aat gtg       288
Tyr Asp Met Lys Ser Leu Leu Thr Glu Ser Met Val Leu Tyr Asn Val
                85                  90                  95
gcg caa gtg ctg ctc aat ggg tgg acg gtg tat gcg att gtg gat gcg       336
Ala Gln Val Leu Leu Asn Gly Trp Thr Val Tyr Ala Ile Val Asp Ala
            100                 105                 110
gtg atg aat aga gac cat ccg ttt att gga agt aga agt ttg gtt ggg       384
Val Met Asn Arg Asp His Pro Phe Ile Gly Ser Arg Ser Leu Val Gly
        115                 120                 125
gcg gcg ttg cat agt ggg agc tcg tat gcg gtg tgg gtt cat tat tgt       432
Ala Ala Leu His Ser Gly Ser Ser Tyr Ala Val Trp Val His Tyr Cys
    130                 135                 140
gat aag tat ttg gag ttc ttt gat acg tat ttt atg gtg ttg agg ggg       480
Asp Lys Tyr Leu Glu Phe Phe Asp Thr Tyr Phe Met Val Leu Arg Gly
145                 150                 155                 160
aaa atg gac cag gtc tcc ttc ctc cac atc tac cac cac acg acc ata       528
Lys Met Asp Gln Val Ser Phe Leu His Ile Tyr His His Thr Thr Ile
                165                 170                 175
gcg tgg gca tgg tgg atc gcc ctc cgc ttc tcc ccc ggt gga gac att       576
Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly Gly Asp Ile
            180                 185                 190
tac ttc ggg gca ctc ctc aac tcc atc atc cac gtc ctc atg tat tcc       624
Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu Met Tyr Ser
        195                 200                 205
tac tac gcc ctt gcc cta ctc aag gtc agt tgt cca tgg aaa cga tac       672
Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp Lys Arg Tyr
    210                 215                 220
ctg act caa gct caa tta ttg caa ttc aca agt gtg gtg gtt tat acg       720
Leu Thr Gln Ala Gln Leu Leu Gln Phe Thr Ser Val Val Val Tyr Thr
225                 230                 235                 240
ggg tgt acg ggt tat act cat tac tat cat acg aag cat gga gcg gat       768
Gly Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His Gly Ala Asp
                245                 250                 255
gag aca cag cct agt tta gga acg tat tat ttc tgt tgt gga gtg cag       816
Glu Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln
            260                 265                 270
gtg ttt gag atg gtt agt ttg ttt gta ctc ttt tcc atc ttt tat aaa       864
Val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
        275                 280                 285
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cga tcc tat tcg aag aag aac aag tca gga gga aag gat agc aag aag        912
Arg Ser Tyr Ser Lys Lys Asn Lys Ser Gly Gly Lys Asp Ser Lys Lys
    290                 295                 300
aat gat gat ggg aat aat gag gat caa tgt cac aag gct atg aag gat        960
Asn Asp Asp Gly Asn Asn Glu Asp Gln Cys His Lys Ala Met Lys Asp
305                 310                 315                 320
ata tcg gag ggt gcg aag gag gtt gtg ggg cat gca gcg aag gat gct       1008
Ile Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala
                325                 330                 335
gga aag ttg gtg gct acg gcg agt aag gct gta aag agg aag gga act       1056
Gly Lys Leu Val Ala Thr Ala Ser Lys Ala Val Lys Arg Lys Gly Thr
            340                 345                 350
cgt gtt act ggt gcc atg tag                                          1077
Arg Val Thr Gly Ala Met
        355

<210> 69
<211> 358
<212> PRT
<213> Thalassiosira pseudonana

<400> 69
Met Cys Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala
1               5                  10                  15
Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Trp Cys
            20                  25                  30
His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu
        35                  40                  45
Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu
    50                  55                  60
Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys
65                  70                  75                  80
Tyr Asp Met Lys Ser Leu Leu Thr Glu Ser Met Val Leu Tyr Asn Val
                85                  90                  95
Ala Gln Val Leu Leu Asn Gly Trp Thr Val Tyr Ala Ile Val Asp Ala
            100                 105                 110
Val Met Asn Arg Asp His Pro Phe Ile Gly Ser Arg Ser Leu Val Gly
        115                 120                 125
Ala Ala Leu His Ser Gly Ser Ser Tyr Ala Val Trp Val His Tyr Cys
    130                 135                 140
Asp Lys Tyr Leu Glu Phe Phe Asp Thr Tyr Phe Met Val Leu Arg Gly
145                 150                 155                 160
Lys Met Asp Gln Val Ser Phe Leu His Ile Tyr His His Thr Thr Ile
                165                 170                 175
Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly Gly Asp Ile
            180                 185                 190
Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu Met Tyr Ser
        195                 200                 205
Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp Lys Arg Tyr
    210                 215                 220
Leu Thr Gln Ala Gln Leu Leu Gln Phe Thr Ser Val Val Val Tyr Thr
225                 230                 235                 240
Gly Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His Gly Ala Asp
                245                 250                 255
Glu Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln
            260                 265                 270
Val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
        275                 280                 285
Arg Ser Tyr Ser Lys Lys Asn Lys Ser Gly Gly Lys Asp Ser Lys Lys
    290                 295                 300
Asn Asp Asp Gly Asn Asn Glu Asp Gln Cys His Lys Ala Met Lys Asp
305                 310                 315                 320
Ile Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala
                325                 330                 335
Gly Lys Leu Val Ala Thr Ala Ser Lys Ala Val Lys Arg Lys Gly Thr
            340                 345                 350
Arg Val Thr Gly Ala Met
        355
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<210> 70
<211> 903
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(903)

<400> 70

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atg agc gcc tcc ggt gcg ctg ctg ccc gcg atc gcg tcc gcc gcg tac        48
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Ser Ala Ala Tyr
1               5                  10                  15
gcg tac gcg acg tac gcc tac gcc ttt gag tgg tcg cac gcg aat ggc        96
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
atc gac aac gtc gac gcg cgc gag tgg atc ggt gcg ctg tcg ttg agg       144
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
ctc ccg gcg atc gcg acg acg atg tac ctg ttg ttc tgc ctg gtc gga       192
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
    50                  55                  60
ccg agg ttg atg gcg aag cgc gag gcg ttc gac ccg aag ggg ttc atg       240
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
ctg gcg tac aat gcg tat cag acg gcg ttc aac gtc gtc gtg ctc ggg       288
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
atg ttc gcg cga gag atc tcg ggg ctg ggg cag ccc gtg tgg ggg tca       336
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
acc atg ccg tgg agc gat aga aaa tcg ttt aag atc ctc ctc ggg gtg       384
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
tgg ttg cac tac aac aac aaa tat ttg gag cta ttg gac act gtg ttc       432
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
atg gtt gcg cgc aag aag acg aag cag ttg agc ttc ttg cac gtt tat       480
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
cat cac gcc ctg ttg atc tgg gcg tgg tgg ttg gtg tgt cac ttg atg       528
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
gcc acg aac gat tgt atc gat gcc tac ttc ggc gcg gcg tgc aac tcg       576
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
ttc att cac atc gtg atg tac tcg tat tat ctc atg tcg gcg ctc ggc       624
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
att cga tgc ccg tgg aag cga tac atc acc cag gct caa atg ctc caa       672
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
    210                 215                 220
ttc gtc att gtc ttc gcg cac gcc gtg ttc gtg ctg cgt cag aag cac       720
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
tgc ccg gtc acc ctt cct tgg gcg caa atg ttc gtc atg acg aac atg       768
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
ctc gtg ctc ttc ggg aac ttc tac ctc aag gcg tac tcg aac aag tcg       816
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
cgc ggc gac ggc gcg agt tcc gtg aaa cca gcc gag acc acg cgc gcg       864
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
ccc agc gtg cga cgc acg cga tct cga aaa att gac taa                   903
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300
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<210> 71
<211> 300
<212> PRT
<213> Ostreococcus tauri

<400> 71
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Ser Ala Ala Tyr
1 5 10 15
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
20 25 30
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
35 40 45
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
50 55 60
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65 70 75 80
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
85 90 95
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
100 105 110
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
115 120 125
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
130 135 140
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145 150 155 160
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
165 170 175
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
180 185 190
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
195 200 205
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
210 215 220
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225 230 235 240
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
245 250 255
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
260 265 270
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
275 280 285
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
290 295 300

<210> 72
<211> 339
<212> PRT
<213> Artificial sequence

<220>
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<222> (3)..(29)
<223> xaa in position 3 to 29 is any amino acid
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<221> Variant
<222> (30)..(43)
<223> xaa in position 30 to 43 is any or no amino acid
<220>
<221> Variant
<222> (45)..(50)
<223> xaa in position 45 to 50 is any amino acid
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<221> Variant
<222> (53)..(73)
<223> xaa in position 53 to 73 is any amino acid

<220>
<221> Variant
<222> (74)..(79)
<223> xaa in position 74 to 79 is any or no amino acid
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<221> Variant
<222> (81)..(82)
<223> xaa in position 81 to 82 is any amino acid
<220>
<221> Variant
<222> (85)..(86)
<223> xaa in position 85 to 86 is any amino acid
<220>
<221> Variant
<222> (88)..(90)
<223> xaa in position 88 to 90 is any amino acid
<220>
<221> Variant
<222> (92)..(110)
<223> xaa in position 92 to 110 is any amino acid
<220>
<221> Variant
<222> (112)..(127)
<223> xaa in position 112 to 127 is any amino acid
<220>
<221> Variant
<222> (128)..(128)
<223> xaa in position 128 is any or no amino acid
<220>
<221> Variant
<222> (130)..(130)
<223> xaa in position 130 is any amino acid
<220>
<221> Variant
<222> (133)..(134)
<223> xaa in position 133 to 134 is any amino acid
<220>
<221> Variant
<222> (137)..(137)
<223> xaa in position 137 is any amino acid
<220>
<221> Variant
<222> (139)..(140)
<223> xaa in position 139 to 140 is any amino acid
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<222> (143)..(144)
<223> xaa in position 143 to 144 is any amino acid
<220>
<221> Variant
<222> (147)..(147)
<223> xaa in position 147 is any amino acid
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<221> Variant
<222> (149)..(149)
<223> xaa in position 149 is any amino acid
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<221> Variant
<222> (151)..(152)
<223> xaa in position 151 to 152 is any amino acid
<220>
<221> Variant
<222> (154)..(154)
<223> xaa in position 154 is any amino acid
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<221> Variant
<222> (159)..(159)
<223> xaa in position 159 is any amino acid

<220>
<221> Variant
<222> (163)..(166)
<223> xaa in position 163 to 166 is any amino acid
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<222> (170)..(179)
<223> xaa in position 170 to 179 is any amino acid
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<221> Variant
<222> (180)..(182)
<223> xaa in position 180 to 182 is any or no amino acid
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<222> (184)..(184)
<223> xaa in position 184 is any amino acid
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<222> (188)..(190)
<223> xaa in position 188 to 190 is any amino acid
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<222> (193)..(193)
<223> xaa in position 193 is any amino acid
<220>
<221> Variant
<222> (196)..(197)
<223> xaa in position 196 to 197 is any amino acid
<220>
<221> Variant
<222> (203)..(206)
<223> xaa in position 203 to 206 is any amino acid
<220>
<221> Variant
<222> (208)..(210)
<223> xaa in position 208 to 210 is any amino acid
<220>
<221> Variant
<222> (217)..(217)
<223> xaa in position 217 is any amino acid
<220>
<221> Variant
<222> (222)..(223)
<223> xaa in position 222 to 223 is any amino acid
<220>
<221> Variant
<222> (226)..(252)
<223> xaa in position 226 to 252 is any amino acid
<220>
<221> Variant
<222> (253)..(267)
<223> xaa in position 253 to 267 is any or no amino acid
<220>
<221> Variant
<222> (269)..(272)
<223> xaa in position 269 to 272 is any amino acid
<220>
<221> Variant
<222> (276)..(277)
<223> xaa in position 276 to 277 is any amino acid
<220>
<221> Variant
<222> (280)..(282)
<223> xaa in position 280 to 282 is any amino acid
<220>
<221> Variant
<222> (284)..(291)
<223> xaa in position 284 to 291 is any amino acid

<220>
<221> Variant
<222> (292)..(324)
<223> xaa in position 292 to 324 is any or no amino acid
<220>
<221> Variant
<222> (326)..(337)
<223> xaa in position 326 to 337 is any amino acid
<220>
<221> Variant
<222> (338)..(338)
<223> xaa in position 338 is any or no amino acid

<400> 72
Leu Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
 1   5   10   15
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
            20   25   30
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Pro xaa xaa xaa xaa
        35   40   45
xaa xaa Tyr Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
    50   55   60
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Met
65   70   75   80
xaa xaa Tyr Asn xaa xaa Gln xaa xaa xaa Asn xaa xaa xaa xaa xaa
                85   90   95
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa
            100   105   110
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
        115   120   125
Trp xaa His Tyr xaa xaa Lys Tyr xaa Glu xaa xaa Asp Thr xaa xaa
    130   135   140
Met Val xaa Arg xaa Lys xaa xaa Gln xaa Ser Phe Leu His xaa Tyr
145   150   155   160
His His xaa xaa xaa xaa Trp Ala Trp xaa xaa xaa xaa xaa xaa xaa
                165   170   175
xaa xaa xaa xaa xaa xaa Asp xaa Tyr Phe Gly xaa xaa xaa Asn Ser
            180   185   190
xaa Ile His xaa xaa Met Tyr Ser Tyr Tyr xaa xaa xaa xaa Leu xaa
        195   200   205
xaa xaa Cys Pro Trp Lys Arg Tyr xaa Thr Gln Ala Gln xaa xaa Gln
    210   215   220
Phe xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
225   230   235   240
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
                245   250   255
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Met xaa xaa xaa xaa
            260   265   270
Val Leu Phe xaa xaa Phe Tyr xaa xaa xaa Tyr xaa xaa xaa xaa xaa
        275   280   285
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
    290   295   300
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
305   310   315   320
xaa xaa xaa xaa Ala xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
                325   330   335
xaa xaa Val

<210> 73
<211> 45
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (4)..(4)

<223> xaa in position 4 is Ala or Gly
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ala or Leu
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Phe or Ile
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is any or no amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any or no amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Leu or Met
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ala or Ser
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Ala or Leu
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Arg or Ser
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Ile or Leu
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Leu or Met
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is Leu or Val
<220>
<221> Variant
<222> (43)..(43)
<223> xaa in position 43 is Cys, Thr or Val
<220>
<221> Variant
<222> (44)..(44)
<223> xaa in position 44 is any amino acid
<220>
<221> Variant
<222> (45)..(45)

<223> xaa in position 45 is Cys or Val

<400> 73
Tyr Phe Gly xaa xaa xaa Asn Ser xaa Ile His xaa Val xaa Met Tyr
  1   5   10   15
Ser Tyr Tyr xaa xaa xaa xaa Leu xaa xaa xaa Cys Pro Trp Lys Arg
            20   25   30
Tyr xaa Thr Gln Ala Gln xaa xaa Gln Phe xaa xaa xaa
        35   40   45

<210> 74
<211> 43
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Leu or Val
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Cys or Asn
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Asp or Asn
<220>
<221> Variant
<222> (9)..(10)
<223> xaa in position 9 to 10 is any or no amino acid
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is any amino acid
<220>
<221> Variant
<222> (13)..(14)
<223> xaa in position 13 to 14 is any or no amino acid
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Phe or Trp
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ala or Leu
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is any amino acid
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Leu or Val
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Ile or Val

<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Ala, Thr or Val
<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is any amino acid
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Ile or Leu
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is any amino acid

<400> 74
Trp xaa His Tyr xaa xaa Lys Tyr xaa xaa Leu xaa xaa xaa Asp Thr
 1   5   10   15
xaa xaa Met Val xaa Arg xaa Lys xaa xaa Gln xaa Ser Phe Leu His
            20   25   30
xaa Tyr His His xaa xaa xaa xaa Trp Ala Trp
        35   40

<210> 75
<211> 37
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any or no amino acid
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is any or no amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Gly, Ser or Thr
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (13)..(14)
<223> xaa in position 13 to 14 is any amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ala or Ser
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Ala or Ser
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Glu or Lys
<220>

<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Ala, Asn or Ser
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Lys or Arg
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ala, Gly or Ser
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is any amino acid
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Glu or Gly
<220>
<221> Variant
<222> (25)..(29)
<223> xaa in position 25 to 29 is any amino acid
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Asn, Pro or Gln
<220>
<221> Variant
<222> (31)..(32)
<223> xaa in position 31 to 32 is Ala, Asp or Glu
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Gly, Ser or Thr
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Asn, Gln or Thr
<220>
<221> Variant
<222> (35)..(36)
<223> xaa in position 35 to 36 is any amino acid
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Ala, Asp or Pro

<400> 75
Met xaa xaa Leu xaa Val Leu Phe xaa xaa Phe Tyr xaa xaa xaa Tyr
  1   5   10   15
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
            20   25   30
xaa xaa xaa xaa xaa
        35

<210> 76
<211> 19
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant

<222> (4)..(7)
<223> xaa in position 4 to 7 is any or no amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Phe, Leu or Val
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Ala, Leu or Val
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any amino acid
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Thr or Val
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Ala, Ile or Leu
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Phe, Ile or Leu

<400> 76
Lys xaa xaa xaa xaa xaa xaa Met xaa xaa Tyr Asn xaa xaa Gln xaa
1 5 10 15
xaa xaa Asn

<210> 77
<211> 1560
<212> DNA
<213> Traustochytrium sp.

<220>
<221> CDS
<222> (1)..(1560)

<400> 77
atg acg gtc ggc tac gac gag gag atc ccg ttc gag cag gtc cgc gcg 48
Met Thr Val Gly Tyr Asp Glu Glu Ile Pro Phe Glu Gln Val Arg Ala
1 5 10 15
cac aac aag ccg gat gac gcc tgg tgc gcg atc cac ggg cac gtg tac 96
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
20 25 30
gat gtg acc aag ttc gcg agc gtg cac ccg ggc ggc gac att atc ctg 144
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
35 40 45
ctg gcc gca ggc aag gag gcc acc gtg ctg tac gag act tac cat gtg 192
Leu Ala Ala Gly Lys Glu Ala Thr Val Leu Tyr Glu Thr Tyr His Val
50 55 60
cgg ggc gtc tcg gac gcg gtg ctg cgc aag tac cgc atc ggc aag ctg 240
Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65 70 75 80
ccg gac ggc caa ggc ggc gcg aac gag aag gaa aag cgg acg ctc tcg 288
Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
85 90 95
ggc ctc tcg tcg gcc tcg tac tac acg tgg aac agc gac ttt tac agg 336
Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
100 105 110

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gta atg cgc gag cgc gtc gtg gct cgg ctc aag gag cgc ggc aag gcc          384
Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
        115                 120                 125
cgc cgc gga ggc tac gag ctc tgg atc aag gcg ttc ctg ctg ctc gtc          432
Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Phe Leu Leu Leu Val
    130                 135                 140
ggc ttc tgg agc tcg ctg tac tgg atg tgc acg ctg gac ccc tcg ttc          480
Gly Phe Trp Ser Ser Leu Tyr Trp Met Cys Thr Leu Asp Pro Ser Phe
145                 150                 155                 160
ggg gcc atc ctg gcc gcc atg tcg ctg ggc gtc ttt gcc gcc ttt gtg          528
Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
                165                 170                 175
ggc acg tgc atc cag cac gac ggc aac cac ggc gcc ttt gcc cag tcg          576
Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
            180                 185                 190
cga tgg gtc aac aag gtt gcc ggg tgg acg ctc gac atg atc ggc gcc          624
Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
        195                 200                 205
agc ggc atg acg tgg gag ttc cag cac gtc ctg ggc cac cat ccg tac          672
Ser Gly Met Thr Trp Glu Phe Gln His Val Leu Gly His His Pro Tyr
    210                 215                 220
acg aac ctg atc gag gag gag aac ggc ctg caa aag gtg agc ggc aag          720
Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
225                 230                 235                 240
aag atg gac acc aag ctg gcc gac cag gag agc gat ccg gac gtc ttt          768
Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
                245                 250                 255
tcc acg tac ccg atg atg cgc ctg cac ccg tgg cac cag aag cgc tgg          816
Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
            260                 265                 270
tac cac cgt ttc cag cac att tac ggc ccc ttc atc ttt ggc ttc atg          864
Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
        275                 280                 285
acc atc aac aag gtg gtc acg cag gac gtc ggt gtg gtg ctc cgc aag          912
Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Leu Arg Lys
    290                 295                 300
cgg ctc ttc cag att gac gcc gag tgc cgg tac gcg agc cca atg tac          960
Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
305                 310                 315                 320
gtg gcg cgt ttc tgg atc atg aag gcg ctc acg gtg ctc tac atg gtg         1008
Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
                325                 330                 335
gcc ctg ccg tgc tac atg cag ggc ccg tgg cac ggc ctc aag ctg ttc         1056
Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
            340                 345                 350
gcg atc gcg cac ttt acg tgc ggc gag gtg ctc gca acc atg ttc att         1104
Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
        355                 360                 365
gtg aac cac atc atc gag ggc gtc tcg tac gct tcc aag gac gcg gtc         1152
Val Asn His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
    370                 375                 380
aag ggc acg atg gcg ccg ccg aag acg atg cac ggc gtg acg ccc atg         1200
Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
385                 390                 395                 400
aac aac acg cgc aag gag gtg gag gcg gag gcg tcc aag tct ggc gcc         1248
Asn Asn Thr Arg Lys Glu Val Glu Ala Glu Ala Ser Lys Ser Gly Ala
                405                 410                 415
gtg gtc aag tca gtc ccg ctc gac gac tgg gcc gcc gtc cag tgc cag         1296
Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val Gln Cys Gln
            420                 425                 430
acc tcg gtg aac tgg agc gtc ggc tcg tgg ttc tgg aat cac ttt tcc         1344
Thr Ser Val Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
        435                 440                 445
ggc ggc ctc aac cac cag att gag cac cac ctg ttc ccc ggg ctc agc         1392
Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
    450                 455                 460
cac gag acg tac tac cac atc cag gac gtc gtt cag tcc acc tgc gcc         1440
His Glu Thr Tyr Tyr His Ile Gln Asp Val Val Gln Ser Thr Cys Ala
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465                 470                 475                 480
gag tac ggc gtc ccg tac cag cac gag cct tcg ctc tgg acc gcg tac      1488
Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
                485                 490                 495
tgg aag atg ctc gag cac ctc cgt cag ctc ggc aat gag gag acc cac      1536
Trp Lys Met Leu Glu His Leu Arg Gln Leu Gly Asn Glu Glu Thr His
            500                 505                 510
gag tcc tgg cag cgc gct gcc tga                                      1560
Glu Ser Trp Gln Arg Ala Ala
        515                 520
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<210> 78
<211> 519
<212> PRT
<213> Traustochytrium sp.

<400> 78

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Met Thr Val Gly Tyr Asp Glu Glu Ile Pro Phe Glu Gln Val Arg Ala
1               5                  10                  15
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
            20                  25                  30
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Val Leu Tyr Glu Thr Tyr His Val
    50                  55                  60
Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65                  70                  75                  80
Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
                85                  90                  95
Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
            100                 105                 110
Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
        115                 120                 125
Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Phe Leu Leu Leu Val
    130                 135                 140
Gly Phe Trp Ser Ser Leu Tyr Trp Met Cys Thr Leu Asp Pro Ser Phe
145                 150                 155                 160
Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
                165                 170                 175
Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
            180                 185                 190
Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
        195                 200                 205
Ser Gly Met Thr Trp Glu Phe Gln His Val Leu Gly His His Pro Tyr
    210                 215                 220
Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
225                 230                 235                 240
Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
                245                 250                 255
Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
            260                 265                 270
Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
        275                 280                 285
Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Leu Arg Lys
    290                 295                 300
Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
305                 310                 315                 320
Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
                325                 330                 335
Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
            340                 345                 350
Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
        355                 360                 365
Val Asn His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
    370                 375                 380
Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
385                 390                 395                 400
Asn Asn Thr Arg Lys Glu Val Glu Ala Glu Ala Ser Lys Ser Gly Ala
```

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                    405                 410                 415
Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val Gln Cys Gln
                420                 425                 430
Thr Ser Val Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
            435                 440                 445
Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
        450                 455                 460
His Glu Thr Tyr Tyr His Ile Gln Asp Val Val Gln Ser Thr Cys Ala
465                 470                 475                 480
Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
                485                 490                 495
Trp Lys Met Leu Glu His Leu Arg Gln Leu Gly Asn Glu Glu Thr His
            500                 505                 510
Glu Ser Trp Gln Arg Ala Ala
        515
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<210> 79
<211> 1560
<212> DNA
<213> Thraustochytrium sp

<220>
<221> CDS
<222> (1)..(1560)

<400> 79

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atg acg gtc ggc tac gac ggg gag atc ccg ttc gag cag gtc cgc gcg        48
Met Thr Val Gly Tyr Asp Gly Glu Ile Pro Phe Glu Gln Val Arg Ala
1               5                   10                  15
cac aac aag ccg gat gac gcc tgg tgc gcg atc cac ggg cac gtg tac        96
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
            20                  25                  30
gat gtg acc aag ttc gcg agc gtg cac ccg ggc ggc gac att atc ctg       144
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
        35                  40                  45
ctg gcc gca ggc aag gat gcc acc gtg ctg tac gag act tac cat gtg       192
Leu Ala Ala Gly Lys Asp Ala Thr Val Leu Tyr Glu Thr Tyr His Val
    50                  55                  60
cgg ggc gtc tcg gac gcg gtg ctg cgc aag tac cgc atc ggc aag ctg       240
Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65                  70                  75                  80
ccg gac ggc caa ggc ggc gcg aac gag aag gaa aag cgg acg ctc tcg       288
Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
                85                  90                  95
ggc ctc tcg tcg gcc tcg tac tac acg tgg aac agc gac ttt tac agg       336
Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
            100                 105                 110
gta atg cgc gag cgc gtc gtg gct cgg ctc aag gag cgc ggc aag gcc       384
Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
        115                 120                 125
cgc cgc gga ggc tac gag ctc tgg atc aag gcg ctc ctg ctg ctc gtc       432
Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Leu Leu Leu Leu Val
    130                 135                 140
ggc ttc tgg agc tcg ctg tgc tgg atg tgc acg ctg gac ccc tcg ttc       480
Gly Phe Trp Ser Ser Leu Cys Trp Met Cys Thr Leu Asp Pro Ser Phe
145                 150                 155                 160
ggg gcc atc ctg gcc gcc atg tcg ctg ggc gtc ttt gcc gcc ttt gtg       528
Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
                165                 170                 175
ggc acg tgc atc cag cac gac ggc aac cac ggc gcc ttt gcc cag tcg       576
Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
            180                 185                 190
cga tgg gtc aac aag gtt gcc ggg tgg acg ctc gac atg atc ggc gcc       624
Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
        195                 200                 205
agc ggc atg acg tgg gag ttc cag cac gcc ctg ggc cac cat ccg tac       672
Ser Gly Met Thr Trp Glu Phe Gln His Ala Leu Gly His His Pro Tyr
    210                 215                 220
```

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acg aac ctg atc gag gag gag aac ggc ctg caa aag gtg agc ggc aag        720
Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
225                 230                 235                 240
aag atg gac acc aag ctg gcc gac cag gag agc gat ccg gac gtc ttt        768
Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
                245                 250                 255
tcc acg tac ccg atg atg cgc ctg cac ccg tgg cac cag aag cgc tgg        816
Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
            260                 265                 270
tac cac cgt ttc cag cac att tac ggc ccc ttc atc ttt ggc ttc atg        864
Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
        275                 280                 285
acc atc aac aag gtg gtc acg cag gac gtc ggt gtg gtg ttc cgc aag        912
Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Phe Arg Lys
    290                 295                 300
cgg ctc ttc cag att gac gcc gag tgc cgg tac gcg agc cca atg tac        960
Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
305                 310                 315                 320
gtg gcg cgt ttc tgg atc atg aag gcg ctc acg gtg ctc tac atg gtg       1008
Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
                325                 330                 335
gcc ctg ccg tgc tac atg cag ggc ccg tgg cac ggc ctc aag ctg ttc       1056
Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
            340                 345                 350
gcg atc gcg cac ttt acg tgc ggc gag gtg ctc gca acc atg ttc att       1104
Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
        355                 360                 365
gtg aac cac gtc atc gag ggc gtc tcg tac gct tcc aag gac gcg gtc       1152
Val Asn His Val Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
    370                 375                 380
aag ggc acg atg gcg ccg ccg aag acg atg cac ggc gtg acg ccc atg       1200
Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
385                 390                 395                 400
aac aac acg cgc aag gag gtg gag gcg gag gcg tcc aag tct ggc gcc       1248
Asn Asn Thr Arg Lys Glu Val Glu Ala Glu Ala Ser Lys Ser Gly Ala
                405                 410                 415
gtg gtc aag tca gtc ccg ctc gac gac tgg gcc gcc gtc cag tgc cag       1296
Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val Gln Cys Gln
            420                 425                 430
acc tcg gcg aac tgg agc gtc ggc tcg tgg ttc tgg aac cac ttt tcc       1344
Thr Ser Ala Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
        435                 440                 445
ggc ggt ctc aac cac cag att gag cac cac ctg ttc ccc ggg ctc agc       1392
Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
    450                 455                 460
cac gag acg tac tac cac atc cag gac gtc gtt cag tcc acc tgc gcc       1440
His Glu Thr Tyr Tyr His Ile Gln Asp Val Val Gln Ser Thr Cys Ala
465                 470                 475                 480
gag tac ggc gtc ccg tac cag cac gag cct tcg ctc tgg acc gcg tac       1488
Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
                485                 490                 495
tgg aag atg ctc gag cac ctc cgt cgg ctc ggc aat gag gag acc cac       1536
Trp Lys Met Leu Glu His Leu Arg Arg Leu Gly Asn Glu Glu Thr His
            500                 505                 510
gag tcc tgg cag cgc gct gcc tga                                      1560
Glu Ser Trp Gln Arg Ala Ala
        515
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<210> 80
<211> 519
<212> PRT
<213> Thraustochytrium sp

<400> 80

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Met Thr Val Gly Tyr Asp Gly Glu Ile Pro Phe Glu Gln Val Arg Ala
1                 5                  10                  15
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
            20                  25                  30
```

```
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
            35                  40                  45
Leu Ala Ala Gly Lys Asp Ala Thr Val Leu Tyr Glu Thr Tyr His Val
        50                  55                  60
Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65                  70                  75                  80
Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
                85                  90                  95
Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
            100                 105                 110
Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
        115                 120                 125
Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Leu Leu Leu Leu Val
    130                 135                 140
Gly Phe Trp Ser Ser Leu Cys Trp Met Cys Thr Leu Asp Pro Ser Phe
145                 150                 155                 160
Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
                165                 170                 175
Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
            180                 185                 190
Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
        195                 200                 205
Ser Gly Met Thr Trp Glu Phe Gln His Ala Leu Gly His His Pro Tyr
    210                 215                 220
Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
225                 230                 235                 240
Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
                245                 250                 255
Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
            260                 265                 270
Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
        275                 280                 285
Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Phe Arg Lys
    290                 295                 300
Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
305                 310                 315                 320
Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
                325                 330                 335
Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
            340                 345                 350
Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
        355                 360                 365
Val Asn His Val Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
    370                 375                 380
Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
385                 390                 395                 400
Asn Asn Thr Arg Lys Glu Val Glu Ala Glu Ala Ser Lys Ser Gly Ala
                405                 410                 415
Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val Gln Cys Gln
            420                 425                 430
Thr Ser Ala Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
        435                 440                 445
Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
    450                 455                 460
His Glu Thr Tyr Tyr His Ile Gln Asp Val Val Gln Ser Thr Cys Ala
465                 470                 475                 480
Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
                485                 490                 495
Trp Lys Met Leu Glu His Leu Arg Arg Leu Gly Asn Glu Glu Thr His
            500                 505                 510
Glu Ser Trp Gln Arg Ala Ala
        515
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<210> 81
<211> 1653
<212> DNA
<213> Thalassiosira pseudonana

<220>
<221> CDS
<222> (1)..(1653)

<400> 81

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atg ggc aac ggc aac ctc cca gca tcc acc gca cag ctc aag tcc acc        48
Met Gly Asn Gly Asn Leu Pro Ala Ser Thr Ala Gln Leu Lys Ser Thr
1               5                  10                  15
tcg aag ccc cag cag caa cat gag cat cgc acc atc tcc aag tcc gag        96
Ser Lys Pro Gln Gln Gln His Glu His Arg Thr Ile Ser Lys Ser Glu
            20                  25                  30
ctc gcc caa cac aac acg ccc aaa tca gca tgg tgt gcc gtc cac tcc       144
Leu Ala Gln His Asn Thr Pro Lys Ser Ala Trp Cys Ala Val His Ser
        35                  40                  45
act ccc gcc acc gac cca tcc cac tcc aac aac aaa caa cac gca cac       192
Thr Pro Ala Thr Asp Pro Ser His Ser Asn Asn Lys Gln His Ala His
    50                  55                  60
cta gtc ctc gac att acc gac ttt gcg tcc cgc cat cca ggg gga gac       240
Leu Val Leu Asp Ile Thr Asp Phe Ala Ser Arg His Pro Gly Gly Asp
65                  70                  75                  80
ctc atc ctc ctc gct tcc ggc aaa gac gcc tcg gtg ctg ttt gaa aca       288
Leu Ile Leu Leu Ala Ser Gly Lys Asp Ala Ser Val Leu Phe Glu Thr
                85                  90                  95
tac cat cca cgt gga gtt ccg acg tct ctc att caa aag ctg cag att       336
Tyr His Pro Arg Gly Val Pro Thr Ser Leu Ile Gln Lys Leu Gln Ile
            100                 105                 110
gga gtg atg gag gag gag gcg ttt cgg gat tcg ttt tac agt tgg act       384
Gly Val Met Glu Glu Glu Ala Phe Arg Asp Ser Phe Tyr Ser Trp Thr
        115                 120                 125
gat tct gac ttt tat act gtg ttg aag agg agg gtt gtg gag cgg ttg       432
Asp Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg Val Val Glu Arg Leu
    130                 135                 140
gag gag agg ggg ttg gac agg agg gga tcg aaa gag att tgg atc aag       480
Glu Glu Arg Gly Leu Asp Arg Arg Gly Ser Lys Glu Ile Trp Ile Lys
145                 150                 155                 160
gct ttg ttc ttg ttg gtt gga ttt tgg tac tgt ttg tac aag atg tat       528
Ala Leu Phe Leu Leu Val Gly Phe Trp Tyr Cys Leu Tyr Lys Met Tyr
                165                 170                 175
act acg tcg gat att gat cag tac ggt att gcc att gcc tat tct att       576
Thr Thr Ser Asp Ile Asp Gln Tyr Gly Ile Ala Ile Ala Tyr Ser Ile
            180                 185                 190
gga atg gga acc ttt gcg gca ttc atc ggc acg tgt att caa cac gat       624
Gly Met Gly Thr Phe Ala Ala Phe Ile Gly Thr Cys Ile Gln His Asp
        195                 200                 205
gga aat cac ggt gca ttc gct cag aac aag tta ctc aac aag ttg gct       672
Gly Asn His Gly Ala Phe Ala Gln Asn Lys Leu Leu Asn Lys Leu Ala
    210                 215                 220
ggg tgg acg ttg gat atg att ggt gcg agt gcg ttt acg tgg gag ctt       720
Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Phe Thr Trp Glu Leu
225                 230                 235                 240
cag cac atg ctg ggg cat cat cca tat acg aat gtg ttg gat ggg gtg       768
Gln His Met Leu Gly His His Pro Tyr Thr Asn Val Leu Asp Gly Val
                245                 250                 255
gag gag gag agg aag gag agg ggg gag gat gtt gct ttg gaa gaa aag       816
Glu Glu Glu Arg Lys Glu Arg Gly Glu Asp Val Ala Leu Glu Glu Lys
            260                 265                 270
gat cag gaa tca gat cca gac gta ttc tcc tcc ttc cct ctc atg aga       864
Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Ser Phe Pro Leu Met Arg
        275                 280                 285
atg cat ccc cac cat aca acc tca tgg tat cat aaa tac caa cac ctc       912
Met His Pro His His Thr Thr Ser Trp Tyr His Lys Tyr Gln His Leu
    290                 295                 300
tac gct cca ccc ctc ttt gca ttg atg aca ctt gcc aaa gta ttc caa       960
Tyr Ala Pro Pro Leu Phe Ala Leu Met Thr Leu Ala Lys Val Phe Gln
305                 310                 315                 320
cag gat ttt gaa gtt gcc aca tcc gga cga tta tat cat att gat gcc      1008
Gln Asp Phe Glu Val Ala Thr Ser Gly Arg Leu Tyr His Ile Asp Ala
                325                 330                 335
```

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aat gta cgt tat ggt tcg gta tgg aat gtc atg agg ttt tgg gct atg        1056
Asn Val Arg Tyr Gly Ser Val Trp Asn Val Met Arg Phe Trp Ala Met
                340                 345                 350
aag gtc att acg atg gga tat atg atg gga tta cca atc tac ttt cat        1104
Lys Val Ile Thr Met Gly Tyr Met Met Gly Leu Pro Ile Tyr Phe His
            355                 360                 365
gga gta ctg agg gga gtt gga ttg ttt gtt att ggg cat ttg gcg tgt        1152
Gly Val Leu Arg Gly Val Gly Leu Phe Val Ile Gly His Leu Ala Cys
        370                 375                 380
gga gag ttg ttg gcg acg atg ttt att gtg aat cac gtc att gag ggt        1200
Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn His Val Ile Glu Gly
385                 390                 395                 400
gtg agt tat gga acg aag gat ttg gtt ggt ggt gcg agt cat gga gat        1248
Val Ser Tyr Gly Thr Lys Asp Leu Val Gly Gly Ala Ser His Gly Asp
                405                 410                 415
gag aag aag att gtc aag cca acg act gta ttg gga gat aca cca atg        1296
Glu Lys Lys Ile Val Lys Pro Thr Thr Val Leu Gly Asp Thr Pro Met
            420                 425                 430
gaa aag act cgc gag gag gca ttg aaa agc aac agc aat aac aac aag        1344
Glu Lys Thr Arg Glu Glu Ala Leu Lys Ser Asn Ser Asn Asn Asn Lys
        435                 440                 445
aag aag gga gag aag aac tcg gta cca tcc gtt cca ttc aac gac tgg        1392
Lys Lys Gly Glu Lys Asn Ser Val Pro Ser Val Pro Phe Asn Asp Trp
    450                 455                 460
gca gca gtc caa tgc cag acc tcc gtg aat tgg tct cca ggc tca tgg        1440
Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser Pro Gly Ser Trp
465                 470                 475                 480
ttc tgg aat cac ttt tct ggg gga ctc tct cat cag att gag cat cac        1488
Phe Trp Asn His Phe Ser Gly Gly Leu Ser His Gln Ile Glu His His
                485                 490                 495
ttg ttc ccc agc att tgt cat aca aac tac tgt cat atc cag gat gtt        1536
Leu Phe Pro Ser Ile Cys His Thr Asn Tyr Cys His Ile Gln Asp Val
            500                 505                 510
gtg gag agt acg tgt gct gag tac gga gtt ccg tat cag agt gag agt        1584
Val Glu Ser Thr Cys Ala Glu Tyr Gly Val Pro Tyr Gln Ser Glu Ser
        515                 520                 525
aat ttg ttt gtt gct tat gga aag atg att agt cat ttg aag ttt ttg        1632
Asn Leu Phe Val Ala Tyr Gly Lys Met Ile Ser His Leu Lys Phe Leu
    530                 535                 540
ggt aaa gcc aag tgt gag tag                                           1653
Gly Lys Ala Lys Cys Glu
545                 550
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<210> 82
<211> 550
<212> PRT
<213> Thalassiosira pseudonana

<400> 82

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Met Gly Asn Gly Asn Leu Pro Ala Ser Thr Ala Gln Leu Lys Ser Thr
1               5                  10                  15
Ser Lys Pro Gln Gln Gln His Glu His Arg Thr Ile Ser Lys Ser Glu
            20                  25                  30
Leu Ala Gln His Asn Thr Pro Lys Ser Ala Trp Cys Ala Val His Ser
        35                  40                  45
Thr Pro Ala Thr Asp Pro Ser His Ser Asn Asn Lys Gln His Ala His
    50                  55                  60
Leu Val Leu Asp Ile Thr Asp Phe Ala Ser Arg His Pro Gly Gly Asp
65                  70                  75                  80
Leu Ile Leu Leu Ala Ser Gly Lys Asp Ala Ser Val Leu Phe Glu Thr
                85                  90                  95
Tyr His Pro Arg Gly Val Pro Thr Ser Leu Ile Gln Lys Leu Gln Ile
            100                 105                 110
Gly Val Met Glu Glu Glu Ala Phe Arg Asp Ser Phe Tyr Ser Trp Thr
        115                 120                 125
Asp Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg Val Val Glu Arg Leu
    130                 135                 140
Glu Glu Arg Gly Leu Asp Arg Arg Gly Ser Lys Glu Ile Trp Ile Lys
```

```
145                 150                 155                 160
Ala Leu Phe Leu Leu Val Gly Phe Trp Tyr Cys Leu Tyr Lys Met Tyr
                165                 170                 175
Thr Thr Ser Asp Ile Asp Gln Tyr Gly Ile Ala Ile Ala Tyr Ser Ile
            180                 185                 190
Gly Met Gly Thr Phe Ala Ala Phe Ile Gly Thr Cys Ile Gln His Asp
        195                 200                 205
Gly Asn His Gly Ala Phe Ala Gln Asn Lys Leu Leu Asn Lys Leu Ala
    210                 215                 220
Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Phe Thr Trp Glu Leu
225                 230                 235                 240
Gln His Met Leu Gly His His Pro Tyr Thr Asn Val Leu Asp Gly Val
                245                 250                 255
Glu Glu Glu Arg Lys Glu Arg Gly Glu Asp Val Ala Leu Glu Glu Lys
            260                 265                 270
Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Ser Phe Pro Leu Met Arg
        275                 280                 285
Met His Pro His His Thr Thr Ser Trp Tyr His Lys Tyr Gln His Leu
    290                 295                 300
Tyr Ala Pro Pro Leu Phe Ala Leu Met Thr Leu Ala Lys Val Phe Gln
305                 310                 315                 320
Gln Asp Phe Glu Val Ala Thr Ser Gly Arg Leu Tyr His Ile Asp Ala
                325                 330                 335
Asn Val Arg Tyr Gly Ser Val Trp Asn Val Met Arg Phe Trp Ala Met
            340                 345                 350
Lys Val Ile Thr Met Gly Tyr Met Met Gly Leu Pro Ile Tyr Phe His
        355                 360                 365
Gly Val Leu Arg Gly Val Gly Leu Phe Val Ile Gly His Leu Ala Cys
    370                 375                 380
Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn His Val Ile Glu Gly
385                 390                 395                 400
Val Ser Tyr Gly Thr Lys Asp Leu Val Gly Gly Ala Ser His Gly Asp
                405                 410                 415
Glu Lys Lys Ile Val Lys Pro Thr Thr Val Leu Gly Asp Thr Pro Met
            420                 425                 430
Glu Lys Thr Arg Glu Glu Ala Leu Lys Ser Asn Ser Asn Asn Asn Lys
        435                 440                 445
Lys Lys Gly Glu Lys Asn Ser Val Pro Ser Val Pro Phe Asn Asp Trp
    450                 455                 460
Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser Pro Gly Ser Trp
465                 470                 475                 480
Phe Trp Asn His Phe Ser Gly Gly Leu Ser His Gln Ile Glu His His
                485                 490                 495
Leu Phe Pro Ser Ile Cys His Thr Asn Tyr Cys His Ile Gln Asp Val
            500                 505                 510
Val Glu Ser Thr Cys Ala Glu Tyr Gly Val Pro Tyr Gln Ser Glu Ser
        515                 520                 525
Asn Leu Phe Val Ala Tyr Gly Lys Met Ile Ser His Leu Lys Phe Leu
    530                 535                 540
Gly Lys Ala Lys Cys Glu
545                 550
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<210> 83
<211> 1626
<212> DNA
<213> Euglena gracilis

<220>
<221> CDS
<222> (1)..(1626)

<400> 83

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atg ttg gtg ctg ttt ggc aat ttc tat gtc aag caa tac tcc caa aag        48
Met Leu Val Leu Phe Gly Asn Phe Tyr Val Lys Gln Tyr Ser Gln Lys
1               5                  10                  15
aac ggc aag ccg gag aac gga gcc acc cct gag aac gga gcg aag ccg        96
Asn Gly Lys Pro Glu Asn Gly Ala Thr Pro Glu Asn Gly Ala Lys Pro
            20                  25                  30
```

```
caa cct tgc gag aac ggc acg gtg gaa aag cga gag aat gac acc gcc          144
Gln Pro Cys Glu Asn Gly Thr Val Glu Lys Arg Glu Asn Asp Thr Ala
            35                  40                  45
aac gtt cgg ccc acc cgt cca gct gga ccc ccg ccg gcc acg tac tac          192
Asn Val Arg Pro Thr Arg Pro Ala Gly Pro Pro Pro Ala Thr Tyr Tyr
    50                  55                  60
gac tcc ctg gca gtg tcg ggg cag ggc aag gag cgg ctg ttc acc acc          240
Asp Ser Leu Ala Val Ser Gly Gln Gly Lys Glu Arg Leu Phe Thr Thr
65                  70                  75                  80
gat gag gtg agg cgg cac atc ctc ccc acc gat ggc tgg ctg acg tgc          288
Asp Glu Val Arg Arg His Ile Leu Pro Thr Asp Gly Trp Leu Thr Cys
                85                  90                  95
cac gaa gga gtc tac gat gtc act gat ttc ctt gcc aag cac cct ggt          336
His Glu Gly Val Tyr Asp Val Thr Asp Phe Leu Ala Lys His Pro Gly
            100                 105                 110
ggc ggt gtc atc acg ctg ggc ctt gga agg gac tgc aca atc ctc atc          384
Gly Gly Val Ile Thr Leu Gly Leu Gly Arg Asp Cys Thr Ile Leu Ile
        115                 120                 125
gag tca tac cac cct gct ggg cgc ccg gac aag gtg atg gag aag tac          432
Glu Ser Tyr His Pro Ala Gly Arg Pro Asp Lys Val Met Glu Lys Tyr
    130                 135                 140
cgc att ggt acg ctg cag gac ccc aag acg ttc tat gct tgg gga gag          480
Arg Ile Gly Thr Leu Gln Asp Pro Lys Thr Phe Tyr Ala Trp Gly Glu
145                 150                 155                 160
tcc gat ttc tac cct gag ttg aag cgc cgg gcc ctt gca agg ctg aag          528
Ser Asp Phe Tyr Pro Glu Leu Lys Arg Arg Ala Leu Ala Arg Leu Lys
                165                 170                 175
gag gct ggt cag gcg cgg cgc ggc ggc ctt ggg gtg aag gcc ctc ctg          576
Glu Ala Gly Gln Ala Arg Arg Gly Gly Leu Gly Val Lys Ala Leu Leu
            180                 185                 190
gtg ctc acc ctc ttc ttc gtg tcg tgg tac atg tgg gtg gcc cac aag          624
Val Leu Thr Leu Phe Phe Val Ser Trp Tyr Met Trp Val Ala His Lys
        195                 200                 205
tcc ttc ctc tgg gcc gcc gtc tgg ggc ttc gcc ggc tcc cac gtc ggg          672
Ser Phe Leu Trp Ala Ala Val Trp Gly Phe Ala Gly Ser His Val Gly
    210                 215                 220
ctg agc atc cag cac gat ggc aac cac ggc gcg ttc agc cgc aac aca          720
Leu Ser Ile Gln His Asp Gly Asn His Gly Ala Phe Ser Arg Asn Thr
225                 230                 235                 240
ctg gtg aac cgc ctg gcg ggg tgg ggc atg gac ttg atc ggc gcg tcg          768
Leu Val Asn Arg Leu Ala Gly Trp Gly Met Asp Leu Ile Gly Ala Ser
                245                 250                 255
tcc acg gtg tgg gag tac cag cac gtc atc ggc cac cac cag tac acc          816
Ser Thr Val Trp Glu Tyr Gln His Val Ile Gly His His Gln Tyr Thr
            260                 265                 270
aac ctc gtg tcg gac acg cta ttc agt ctg cct gag aac gat ccg gac          864
Asn Leu Val Ser Asp Thr Leu Phe Ser Leu Pro Glu Asn Asp Pro Asp
        275                 280                 285
gtc ttc tcc agc tac ccg ctg atg cgc atg cac ccg gat acg gcg tgg          912
Val Phe Ser Ser Tyr Pro Leu Met Arg Met His Pro Asp Thr Ala Trp
    290                 295                 300
cag ccg cac cac cgc ttc cag cac ctg ttc gcg ttc cca ctg ttc gcc          960
Gln Pro His His Arg Phe Gln His Leu Phe Ala Phe Pro Leu Phe Ala
305                 310                 315                 320
ctg atg aca atc agc aag gtg ctg acc agc gat ttc gct gtc tgc ctc         1008
Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu
                325                 330                 335
agc atg aag aag ggg tcc atc gac tgc tcc tcc agg ctc gtc cca ctg         1056
Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu
            340                 345                 350
gag ggg cag ctg ctg ttc tgg ggg gcc aag ctg gcg aac ttc ctg ttg         1104
Glu Gly Gln Leu Leu Phe Trp Gly Ala Lys Leu Ala Asn Phe Leu Leu
        355                 360                 365
cag att gtg ttg cca tgc tac ctc cac ggg aca gct atg ggc ctg gcc         1152
Gln Ile Val Leu Pro Cys Tyr Leu His Gly Thr Ala Met Gly Leu Ala
    370                 375                 380
ctc ttc tct gtt gct cac ctt gtg tcg ggg gag tac ctc gcg atc tgc         1200
Leu Phe Ser Val Ala His Leu Val Ser Gly Glu Tyr Leu Ala Ile Cys
```

```
385                 390                 395                 400
ttc atc atc aac cac atc agc gag tct tgt gag ttt atg aat aca agc      1248
Phe Ile Ile Asn His Ile Ser Glu Ser Cys Glu Phe Met Asn Thr Ser
                405                 410                 415
ttt caa acc gcc gcc cgg agg aca gag atg ctt cag gca gca cat cag      1296
Phe Gln Thr Ala Ala Arg Arg Thr Glu Met Leu Gln Ala Ala His Gln
            420                 425                 430
gca gcg gag gcc aag aag gtg aag ccc acc cct cca ccg aac gat tgg      1344
Ala Ala Glu Ala Lys Lys Val Lys Pro Thr Pro Pro Pro Asn Asp Trp
        435                 440                 445
gct gtg aca cag gtc caa tgc tgc gtg aat tgg aga tca ggt ggc gtg      1392
Ala Val Thr Gln Val Gln Cys Cys Val Asn Trp Arg Ser Gly Gly Val
    450                 455                 460
ttg gcc aat cac ctc tct gga ggc ttg aac cac cag atc gag cat cat      1440
Leu Ala Asn His Leu Ser Gly Gly Leu Asn His Gln Ile Glu His His
465                 470                 475                 480
ctg ttc ccc agc atc tcg cat gcc aac tac ccc acc atc gcc cct gtt      1488
Leu Phe Pro Ser Ile Ser His Ala Asn Tyr Pro Thr Ile Ala Pro Val
                485                 490                 495
gtg aag gag gtg tgc gag gag tac ggg ttg ccg tac aag aat tac gtc      1536
Val Lys Glu Val Cys Glu Glu Tyr Gly Leu Pro Tyr Lys Asn Tyr Val
            500                 505                 510
acg ttc tgg gat gca gtc tgt ggc atg gtt cag cac ctc cgg ttg atg      1584
Thr Phe Trp Asp Ala Val Cys Gly Met Val Gln His Leu Arg Leu Met
        515                 520                 525
ggt gct cca ccg gtg cca acg aac ggg gac aaa aag tca taa              1626
Gly Ala Pro Pro Val Pro Thr Asn Gly Asp Lys Lys Ser
    530                 535                 540
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<210> 84
<211> 541
<212> PRT
<213> Euglena gracilis

<400> 84

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Met Leu Val Leu Phe Gly Asn Phe Tyr Val Lys Gln Tyr Ser Gln Lys
1               5                  10                  15
Asn Gly Lys Pro Glu Asn Gly Ala Thr Pro Glu Asn Gly Ala Lys Pro
            20                  25                  30
Gln Pro Cys Glu Asn Gly Thr Val Glu Lys Arg Glu Asn Asp Thr Ala
        35                  40                  45
Asn Val Arg Pro Thr Arg Pro Ala Gly Pro Pro Pro Ala Thr Tyr Tyr
    50                  55                  60
Asp Ser Leu Ala Val Ser Gly Gln Gly Lys Glu Arg Leu Phe Thr Thr
65                  70                  75                  80
Asp Glu Val Arg Arg His Ile Leu Pro Thr Asp Gly Trp Leu Thr Cys
                85                  90                  95
His Glu Gly Val Tyr Asp Val Thr Asp Phe Leu Ala Lys His Pro Gly
            100                 105                 110
Gly Gly Val Ile Thr Leu Gly Leu Gly Arg Asp Cys Thr Ile Leu Ile
        115                 120                 125
Glu Ser Tyr His Pro Ala Gly Arg Pro Asp Lys Val Met Glu Lys Tyr
    130                 135                 140
Arg Ile Gly Thr Leu Gln Asp Pro Lys Thr Phe Tyr Ala Trp Gly Glu
145                 150                 155                 160
Ser Asp Phe Tyr Pro Glu Leu Lys Arg Arg Ala Leu Ala Arg Leu Lys
                165                 170                 175
Glu Ala Gly Gln Ala Arg Arg Gly Gly Leu Gly Val Lys Ala Leu Leu
            180                 185                 190
Val Leu Thr Leu Phe Phe Val Ser Trp Tyr Met Trp Val Ala His Lys
        195                 200                 205
Ser Phe Leu Trp Ala Ala Val Trp Gly Phe Ala Gly Ser His Val Gly
    210                 215                 220
Leu Ser Ile Gln His Asp Gly Asn His Gly Ala Phe Ser Arg Asn Thr
225                 230                 235                 240
Leu Val Asn Arg Leu Ala Gly Trp Gly Met Asp Leu Ile Gly Ala Ser
                245                 250                 255
Ser Thr Val Trp Glu Tyr Gln His Val Ile Gly His His Gln Tyr Thr
```

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                260                 265                 270
Asn Leu Val Ser Asp Thr Leu Phe Ser Leu Pro Glu Asn Asp Pro Asp
            275                 280                 285
Val Phe Ser Ser Tyr Pro Leu Met Arg Met His Pro Asp Thr Ala Trp
    290                 295                 300
Gln Pro His His Arg Phe Gln His Leu Phe Ala Phe Pro Leu Phe Ala
305                 310                 315                 320
Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu
                325                 330                 335
Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu
            340                 345                 350
Glu Gly Gln Leu Leu Phe Trp Gly Ala Lys Leu Ala Asn Phe Leu Leu
        355                 360                 365
Gln Ile Val Leu Pro Cys Tyr Leu His Gly Thr Ala Met Gly Leu Ala
    370                 375                 380
Leu Phe Ser Val Ala His Leu Val Ser Gly Glu Tyr Leu Ala Ile Cys
385                 390                 395                 400
Phe Ile Ile Asn His Ile Ser Glu Ser Cys Glu Phe Met Asn Thr Ser
                405                 410                 415
Phe Gln Thr Ala Ala Arg Arg Thr Glu Met Leu Gln Ala Ala His Gln
            420                 425                 430
Ala Ala Glu Ala Lys Lys Val Lys Pro Thr Pro Pro Pro Asn Asp Trp
        435                 440                 445
Ala Val Thr Gln Val Gln Cys Cys Val Asn Trp Arg Ser Gly Gly Val
    450                 455                 460
Leu Ala Asn His Leu Ser Gly Gly Leu Asn His Gln Ile Glu His His
465                 470                 475                 480
Leu Phe Pro Ser Ile Ser His Ala Asn Tyr Pro Thr Ile Ala Pro Val
                485                 490                 495
Val Lys Glu Val Cys Glu Glu Tyr Gly Leu Pro Tyr Lys Asn Tyr Val
            500                 505                 510
Thr Phe Trp Asp Ala Val Cys Gly Met Val Gln His Leu Arg Leu Met
        515                 520                 525
Gly Ala Pro Pro Val Pro Thr Asn Gly Asp Lys Lys Ser
    530                 535                 540

<210> 85
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<220>
<221> CDS
<222> (1)..(1548)

<400> 85
atg acg gtc ggg ttt gac gaa acg gtg act atg gac acg gtc cgc aac      48
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                  10                  15
cac aac atg ccg gac gac gcc tgg tgc gcg atc cac ggc acc gtg tac      96
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
gac atc acc aag ttc agc aag gtg cac ccc ggc ggg gac atc atc atg     144
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
ctg gcc gct ggc aag gag gcc acc atc ctg ttc gag acc tac cac atc     192
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
aag ggc gtc ccg gac gcg gtg ctg cgc aag tac aag gtc ggc aag ctc     240
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
ccc cag ggc aag aag ggc gaa acg agc cac atg ccc acc ggg ctc gac     288
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
tcg gcc tcc tac tac tcg tgg gac agc gag ttt tac agg gtg ctc cgc     336
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
gag cgc gtc gcc aag aag ctg gcc gag ccc ggc ctc atg cag cgc gcg     384
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Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
            115                 120                 125
cgc atg gag ctc tgg gcc aag gcg atc ttc ctc ctg gca ggt ttc tgg      432
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
ggc tcc ctt tac gcc atg tgc gtg cta gac ccg cac ggc ggt gcc atg      480
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
gta gcc gcc gtt acg ctc ggc gtg ttc gct gcc ttt gtc gga act tgc      528
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
atc cag cac gac ggc agc cac ggc gcc ttc tcc aag tcg cga ttc atg      576
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
aac aag gcg gcg ggc tgg acc ctc gac atg atc ggc gcg agt gcg atg      624
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
acc tgg gag atg cag cac gtt ctt ggc cac cac ccg tac acc aac ctc      672
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
atc gag atg gag aac ggt ttg gcc aag gtc aag ggc gcc gac gtc gac      720
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
ccg aag aag gtc gac cag gag agc gac ccg gac gtc ttc agt acg tac      768
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
ccg atg ctt cgc ctg cac ccg tgg cac cgc cag cgg ttt tac cac aag      816
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
ttc cag cac ctg tac gcc ccg ttt atc ttt ggg tct atg acg att aac      864
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn
        275                 280                 285
aag gtg att tcc cag gat gtc ggg gtt gtg ctg cgc aag cgc ctg ttc      912
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
cag atc gac gcc aac tgc cgg tat ggc agc ccc tgg tac gtg gcc cgc      960
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
ttc tgg atc atg aag ctc ctc acc acg ctc tac atg gtg gcg ctt ccc     1008
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
atg tac atg cag ggg cct gct cag ggc ttg aag ctt ttc ttc atg gcc     1056
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
cac ttc acc tgc gga gag gtc ctc gcc acc atg ttt att gtc aac cac     1104
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
atc atc gag ggc gtc agc tac gct tcc aag gac gcg gtc aag ggc gtc     1152
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
atg gct ccg ccg cgc act gtg cac ggt gtc acc ccg atg cag gtg acg     1200
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
caa aag gcg ctc agt gcg gcc gag tcg gcc aag tcg gac gcc gac aag     1248
Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys
                405                 410                 415
acg acc atg atc ccc ctc aac gac tgg gcc gct gtg cag tgc cag acc     1296
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
tct gtg aac tgg gct gtc ggg tcg tgg ttt tgg aac cac ttt tcg ggc     1344
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
ggc ctc aac cac cag att gag cac cac tgc ttc ccc caa aac ccc cac     1392
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
acg gtc aac gtc tac atc tcg ggc atc gtc aag gag acc tgc gaa gaa     1440
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
```

```
tac ggc gtg ccg tac cag gct gag atc agc ctc ttc tct gcc tat ttc        1488
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
aag atg ctg tcg cac ctc cgc acg ctc ggc aac gag gac ctc acg gcc        1536
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
tgg tcc acg tga                                                    1548
Trp Ser Thr
        515
```

<210> 86
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 86

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Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                  10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys
                405                 410                 415
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Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
                420                 425                 430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
            435                 440                 445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
        450                 455                 460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
Trp Ser Thr
        515

<210> 87
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<220>
<221> CDS
<222> (1)..(1548)

<400> 87
atg acg gtc ggg ttt gac gaa acg gtg act atg gac acg gtc cgc aac        48
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                   10                  15
cac aac atg ccg gac gac gcc tgg tgc gcg atc cac ggc acc gtg tac        96
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
gac atc acc aag ttc agc aag gtg cac ccc ggc ggg gac atc atc atg       144
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
ctg gcc gct ggc aag gag gcc acc atc ctg ttc gag acg tac cac atc       192
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
aag ggc gtt ccg gac gcg gtg ctg cgc aag tac aag gtc ggc aag ctc       240
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
ccc cag ggc aag aag ggc gaa acg agc cac atg ccc acc ggg ctc gac       288
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
tcg gcc ttc tac tac tcg tgg gac agc gag ttt tac agg gtg ctc cgc       336
Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
gag cgc gtc gcc aag aag ctg gcc gag ccc ggc ctc atg cag cgc gcg       384
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
cgc atg gag ctc tgg gcc aag gcg atc ttc ctc ctg gca ggt ttc tgg       432
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
ggc tcc ctt tac gcc atg tgc gtg cta gac ccg cac ggc ggt gcc atg       480
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
gta gcc gcc gtt acg ctc ggc gtg ttc gct gcc ttt gtc gga act tgc       528
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
atc cag cac gac ggc agc cac ggc gcc ttc tcc aag tcg cga ttc atg       576
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
aac aag gcg gcg ggc tgg acc ctc gac atg atc ggc gcg agc gcg atg       624
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
acc tgg gag atg cag cac gtt ctt ggc cac cac ccg tac acc aac ctc       672
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
atc gag atg gag aac ggt ttg gcc aag gtc aag ggc gcc gac gtc gac       720
```

```
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
ccg aag aag gtc gac cag gag agc gac ccg gac gtc ttc agt acg tac        768
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
ccg atg ctt cgc ctg cac ccg tgg cac cgc cag cgg ttt tac cac aag        816
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
ttc cag cac ctg tac gcc ccg ttt atc ttt ggg ttt atg acg att aac        864
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
aag gtg att tcc cag gat gtc ggg gtt gtg ctg cgc aag cgc ctg ttc        912
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
cag atc gac gcc aac tgc cgg tat ggc agc ccc tgg tac gtg gcc cgc        960
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
ttc tgg atc atg aag ctc ctc acc acg ctc tac atg gtg gcg ctt ccc       1008
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
atg tac atg cag ggg cct gct cag ggc ttg aag ctt ttc ttc atg gcc       1056
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
cac ttc acc tgc gga gag gtc ctc gcc acc atg ttt att gtc aac cac       1104
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
atc atc gag ggc gtc agc tac gct tcc aag gac gcg gtc aag ggc gtc       1152
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
atg gct ccg ccg cgc act gtg cac ggt gtc acc ccg atg cag gtg acg       1200
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
caa aag gcg ctc agt gcg gcc gag tcg acc aag tcg gac gcc gac aag       1248
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
acg acc atg atc ccc ctc aac gac tgg gcc gct gtg cag tgc cag acc       1296
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
tct gtg aac tgg gct gtc ggg tcg tgg ttt tgg aac cac ttt tcg ggc       1344
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
ggc ctc aac cac cag att gag cac cac tgc ttc ccc caa aac ccc cac       1392
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
acg gtc aac gtc tac atc tca ggc atc gtc aag gag acc tgc gaa gaa       1440
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
tac ggc gtg ccg tac cag gct gag atc agc ctc ttc tct gcc tat ttc       1488
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
aag atg ctg tcg cac ctc cgc acg ctc ggc aac gag gac ctc acg gcc       1536
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
tgg tcc acg tga                                                      1548
Trp Ser Thr
        515

<210> 88
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 88
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1                   5                   10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
```

```
          35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
     50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
           100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
          115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
     130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
           180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
          195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
     210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
           260                 265                 270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
          275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
     290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
           340                 345                 350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
          355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
     370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
           420                 425                 430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
          435                 440                 445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
     450                 455                 460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
           500                 505                 510
Trp Ser Thr
          515
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<210> 89
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<220>

<221> CDS
<222> (1)..(1548)

<400> 89

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atg acg gtc ggg ttt gac gaa acg gtg act atg gac acg gtc cgc aac        48
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                  10                  15
cac aac atg ccg gac gac gcc tgg tgc gcg atc cac ggc acc gtg tac        96
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
gac atc acc aag ttc agc aag gtg cac ccc ggc ggg gac atc atc atg       144
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
ctg gcc gct ggc aag gag gcc acc atc ctg ttc gag acg tac cac atc       192
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
aag ggc gtc ccg gac gcg gtg ctg cgc aag tac aag gtc ggc aag ctc       240
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
ccc cag ggc aag aag ggc gaa acg agc cac atg ccc acc ggg ctc gac       288
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
tcg gcc tcc tac tac tcg tgg gac agc gag ttt tac agg gtg ctc cgc       336
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
gag cgc gtc gcc aag aag ctg gcc gag ccc ggc ctc atg cag cgc gcg       384
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
cgc atg gag ctc tgg gcc aag gcg atc ttc ctc ctg gca ggt ttc tgg       432
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
ggc tcc ctt tac gcc atg tgc gtg cta gac ccg cac ggc ggt gcc atg       480
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
gta gcc gcc gtt acg ctc ggc gtg ttc gct gcc ttt gtc gga act tgc       528
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
atc cag cac gac ggc agc cac ggc gcc ttc tcc aag tcg cga ttc atg       576
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
aac aag gcg gcg ggc tgg acc ctc gac atg atc ggc gcg agc gcg atg       624
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
acc tgg gag atg cag cac gtt ctt ggc cac cac ccg tac acc aac ctc       672
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
atc gag atg gag aac ggt ttg gcc aag gtc aag ggc gcc gac gtc gac       720
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
ccg aag aag gtc gac cag gag agc gac ccg gac gtc ttc agt acg tac       768
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
ccg atg ctt cgc ctg cac ccg tgg cac cgc cag cgg ttt tac cac aag       816
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
ttc cag cac ctg tac gcc ccg ttt atc ttt ggg ttt atg acg att aac       864
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
aag gtg att tcc cag gat gtc ggg gtt gtg ctg cgc aag cgc ctg ttc       912
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
cag atc gac gcc aac tgc cgg tat ggc agc ccc tgg tac gtg gcc cgc       960
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
ttc tgg atc atg aag ctc ctc acc acg ctc tac acg gtg gcg ctt ccc      1008
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Thr Val Ala Leu Pro
                325                 330                 335
atg tac atg cag ggg cct gct cag ggc ttg aag ctt ttc ttc atg gcc      1056
```

```
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
                340                 345                 350
cac ttc acc tgc gga gag gtc ctc gcc acc atg ttt att gtc aac cac        1104
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
            355                 360                 365
atc atc gag ggc gtc agc tac gct tcc aag gac gcg gtc aag ggc gtc        1152
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
        370                 375                 380
atg gct ccg ccg cgc act gtg cac ggt gtc acc ccg atg cag gtg acg        1200
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
caa aag gcg ctc agt gcg gcc gag tcg acc aag tcg gac gcc gac aag        1248
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
acg acc atg atc ccc ctc aac gac tgg gcc gct gtg cag tgc cag acc        1296
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
tct gtg aac tgg gct gtc ggg tcg tgg ttt tgg aac cac ttt tcg ggc        1344
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
ggc ctc aac cac cag att gag cac cac tgc ttc ccc caa aac ccc cac        1392
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
acg gtc aac gtc tac atc tcg ggc atc gtc aag gag acc tgc gaa gaa        1440
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
tac ggc gtg ccg tac cag gct gag atc agc ctc ttc tct gcc tat ttc        1488
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
aag atg ctg tcg cac ctc cgc acg ctc ggc aac gag gac ctc acg gcc        1536
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
agg tcc acg tga                                                     1548
Arg Ser Thr
        515
```

<210> 90
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 90

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Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                  10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
```

```
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Thr Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
Arg Ser Thr
        515
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<210> 91
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<220>
<221> CDS
<222> (1)..(1548)

<400> 91

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atg acg gtc ggg ttt gac gaa acg gtg act atg gac acg gtc cgc aac        48
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                  10                  15
cac aac atg ccg gac gac gcc tgg tgc gcg atc cac ggc acc gtg tac        96
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
gac atc acc aag ttc agc aag gtg cac ccc ggc ggg gac atc atc atg       144
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
ctg gcc gct ggc aag gag gcc acc atc ctg ttc gag acc tac cac atc       192
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
aag ggc gtc ccg gac gcg gtg ctg cgc aag tac aag gtc ggc aag ctc       240
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
ccc cag ggc aag aag ggc gaa acg agc cac atg ccc acc ggg ctc gac       288
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
```

```
                                85                  90                  95
tcg gcc tcc tac tac tcg tgg gac agc gag ttt tac agg gtg ctc cgc          336
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
                            100                 105                 110
gag cgc gtc gcc aag aag ctg gcc gag ccc ggc ctc atg cag cgc gcg          384
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
cgc atg gag ctc tgg gcc aag gcg atc ttc ctc ctg gca ggt ttc tgg          432
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
ggc tcc ctt tac gcc atg tgc gtg cta gac ccg cac ggc ggt gcc atg          480
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
gta gcc gcc gtt acg ctc ggc gtg ttc gct gcc ttt gtc gga act tgc          528
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
atc cag cac gac ggc agc cac ggc gcc ttc tcc aag tcg cga ttc atg          576
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
aac aag gcg gcg ggc tgg acc ctc gac atg atc ggc gcg agc gcg atg          624
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
acc tgg gag atg cag cac gtt ctt ggt cac cac ccg tac acc aac ctc          672
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
atc gag atg gag aac ggt ttg gcc aag gtc aag ggc gcc gac gtc gac          720
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
ccg aag aag gtc gac cag gag agc gac ccg gac gtc ttc agt acg tac          768
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
ccg atg ctt cgc ctg cac ccg tgg cac cgc cag cgg ttt tac cac aag          816
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
ttc cag cac ctg tac gcc ccg ctt atc ttt ggg ttt atg acg att aac          864
Phe Gln His Leu Tyr Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
aag gtg att tcc cag gat gtc ggg gtt gtg ctg cgc aag cgc ctg ttc          912
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
cag atc gac gcc aac tgc cgg tat ggc agc ccc tgg aac gtg gcc cgc          960
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg
305                 310                 315                 320
ttc tgg atc atg aag ctc ctc acc acg ctc tac atg gtg gcg ctt ccc         1008
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
atg tac atg cag ggg cct gct cag ggc ttg aag ctt ttc ttc atg gcc         1056
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
cac ttc acc tgc gga gag gtc ctc gcc acc atg ttt att gtc aac cac         1104
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
atc atc gag ggc gtc agc tac gct tcc aag gac gcg gtc aag ggc gtc         1152
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
atg gct ccg ccg cgc act gtg cac ggt gtc acc ccg atg cag gtg acg         1200
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
caa aag gcg ctc agt gcg gcc gag tcg acc aag tcg gac gcc gac aag         1248
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
acg acc atg atc ccc ctc aac gac tgg gcc gct gtg cag tgc cag acc         1296
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
tct gtg aac tgg gct gtc ggg tcg tgg ttt tgg aac cac ttt tcg ggc         1344
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
ggc cta aac cac cag att gag cac cac tgc ttc ccc caa aac ccc cac         1392
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Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
acg gtc aac gtc tac atc tcg ggc atc gtc aag gag acc tgc gaa gaa      1440
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
tac ggc gtg ccg tac cag gct gag atc agc ctc ttc tct gcc tat ttc      1488
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
aag atg ctg tcg cac ctc cgc acg ctc ggc aac gag gac ctc acg gcc      1536
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
tgg tcc acg tga                                                     1548
Trp Ser Thr
        515

<210> 92
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 92
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                  10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
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    370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
Trp Ser Thr
        515

<210> 93
<211> 1560
<212> DNA
<213> Thraustochytrium sp

<220>
<221> CDS
<222> (1)..(1560)

<400> 93
atg acg gtc ggc tac gac gag gag atc ccg ttc gag cag gtc cgc gcg        48
Met Thr Val Gly Tyr Asp Glu Glu Ile Pro Phe Glu Gln Val Arg Ala
1               5                   10                  15
cac aac aag ccg gat gac gcc tgg tgc gcg atc cac ggg cac gtg tac        96
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
            20                  25                  30
gat gtg acc aag ttc gcg agc gtg cac ccg ggc ggc gac att atc ctg       144
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
        35                  40                  45
ctg gcc gca ggc aag gag gcc acc gtg ctg tac gag act tac cat gtg       192
Leu Ala Ala Gly Lys Glu Ala Thr Val Leu Tyr Glu Thr Tyr His Val
    50                  55                  60
cgg ggc gtc tcg gac gcg gtg ctg cgc aag tac cgc atc ggc aag ctg       240
Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65                  70                  75                  80
ccg gac ggc caa ggc ggc gcg aac gag aag gaa aag cgg acg ctc tcg       288
Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
                85                  90                  95
ggc ctc tcg tcg gcc tcg tac tac acg tgg aac agc gac ttt tac agg       336
Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
            100                 105                 110
gta atg cgc gag cgc gtc gtg gct cgg ctc aag gag cgc ggc aag gcc       384
Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
        115                 120                 125
cgc cgc gga ggc tac gag ctc tgg atc aag gcg ttc ctg ctg ctc gtc       432
Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Phe Leu Leu Leu Val
    130                 135                 140
ggc ttc tgg agc tcg ctg tac tgg atg tgc acg ctg gac ccc tcg ttc       480
Gly Phe Trp Ser Ser Leu Tyr Trp Met Cys Thr Leu Asp Pro Ser Phe
145                 150                 155                 160
ggg gcc atc ctg gcc gcc atg tcg ctg ggc gtc ttt gcc gcc ttt gtg       528
Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
                165                 170                 175
ggc acg tgc atc cag cac gac ggc aac cac ggc gcc ttt gcc cag tcg       576
Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
            180                 185                 190
cga tgg gtc aac aag gtt gcc ggg tgg acg ctc gac atg atc ggc gcc       624
Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
```

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          195                 200                 205
agc ggc atg acg tgg gag ttc cag cac gtc ctg ggc cac cat ccg tac        672
Ser Gly Met Thr Trp Glu Phe Gln His Val Leu Gly His His Pro Tyr
    210                 215                 220
acg aac ctg atc gag gag gag aac ggc ctg caa aag gtg agc ggc aag        720
Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
225                 230                 235                 240
aag atg gac acc aag ctg gcc gac cag gag agc gat ccg gac gtc ttt        768
Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
                245                 250                 255
tcc acg tac ccg atg atg cgc ctg cac ccg tgg cac cag aag cgc tgg        816
Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
            260                 265                 270
tac cac cgt ttc cag cac att tac ggc ccc ttc atc ttt ggc ttc atg        864
Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
        275                 280                 285
acc atc aac aag gtg gtc acg cag gac gtc ggt gtg gtg ctc cgc aag        912
Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Leu Arg Lys
    290                 295                 300
cgg ctc ttc cag att gac gcc gag tgc cgg tac gcg agc cca atg tac        960
Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
305                 310                 315                 320
gtg gcg cgt ttc tgg atc atg aag gcg ctc acg gtg ctc tac atg gtg       1008
Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
                325                 330                 335
gcc ctg ccg tgc tac atg cag ggc ccg tgg cac ggc ctc aag ctg ttc       1056
Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
            340                 345                 350
gcg atc gcg cac ttt acg tgc ggc gag gtg ctc gca acc atg ttc att       1104
Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
        355                 360                 365
gtg aac cac atc atc gag ggc gtc tcg tac gct tcc aag gac gcg gtc       1152
Val Asn His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
    370                 375                 380
aag ggc acg atg gcg ccg ccg aag acg atg cac ggc gtg acg ccc atg       1200
Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
385                 390                 395                 400
aac aac acg cgc aag gag gtg gag gcg gag gcg tcc aag tct ggc gcc       1248
Asn Asn Thr Arg Lys Glu Val Glu Ala Glu Ala Ser Lys Ser Gly Ala
                405                 410                 415
gtg gtc aag tca gtc ccg ctc gac gac tgg gcc gtc gtc cag tgc cag       1296
Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Val Val Gln Cys Gln
            420                 425                 430
acc tcg gtg aac tgg agc gtc ggc tcg tgg ttc tgg aat cac ttt tcc       1344
Thr Ser Val Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
        435                 440                 445
ggc ggc ctc aac cac cag att gag cac cac ctg ttc ccc ggr ctc agc       1392
Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
    450                 455                 460
cac gag acg tac tac cac att cag gac gtc ttt cag tcc acc tgc gcc       1440
His Glu Thr Tyr Tyr His Ile Gln Asp Val Phe Gln Ser Thr Cys Ala
465                 470                 475                 480
gag tac ggc gtc ccg tac cag cac gag cct tcg ctc tgg acc gcg tac       1488
Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
                485                 490                 495
tgg aag atg ctc gag cac ctc cgt cag ctc ggc aat gag gag acc cac       1536
Trp Lys Met Leu Glu His Leu Arg Gln Leu Gly Asn Glu Glu Thr His
            500                 505                 510
gag tcc tgg cag cgc gct gcc tga                                      1560
Glu Ser Trp Gln Arg Ala Ala
        515
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<210> 94
<211> 519
<212> PRT
<213> Thraustochytrium sp

<400> 94

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Met Thr Val Gly Tyr Asp Glu Glu Ile Pro Phe Glu Gln Val Arg Ala
1               5                  10                  15
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
            20                  25                  30
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Val Leu Tyr Glu Thr Tyr His Val
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Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
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Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
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Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
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Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
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Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Phe Leu Leu Leu Val
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Gly Phe Trp Ser Ser Leu Tyr Trp Met Cys Thr Leu Asp Pro Ser Phe
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Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
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Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
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Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
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Ser Gly Met Thr Trp Glu Phe Gln His Val Leu Gly His His Pro Tyr
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Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
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Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
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Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
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Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
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Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Leu Arg Lys
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Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
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Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
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Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
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Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
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Val Asn His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
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Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
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Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Val Val Gln Cys Gln
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Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
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His Glu Thr Tyr Tyr His Ile Gln Asp Val Phe Gln Ser Thr Cys Ala
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Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
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Val Arg xaa His Asn xaa Pro xaa Asp Ala Trp Cys Ala xaa His xaa
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xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
65                  70                  75                  80
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xaa Ile xaa Leu Ala xaa Gly Lys xaa Ala Thr xaa Leu xaa Glu Thr
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Tyr His xaa xaa Gly Val xaa Asp xaa Val xaa xaa Lys Tyr xaa xaa
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Gly xaa Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
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xaa Phe Tyr xaa Val xaa xaa xaa Arg Val xaa xaa xaa Leu xaa Glu
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xaa Gly xaa xaa xaa Arg xaa xaa xaa Glu Leu Trp xaa Lys Ala xaa
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xaa Leu Leu xaa Gly Phe Trp xaa Ser Leu Tyr xaa Met xaa xaa xaa
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xaa xaa xaa Asp xaa xaa xaa xaa Ala xaa xaa xaa Ala xaa xaa xaa
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Gly xaa Phe Ala Ala Phe Val Gly Thr Cys Ile Gln His Asp Gly xaa
225                 230                 235                 240
His Gly Ala Phe xaa xaa xaa xaa xaa xaa Asn Lys xaa Ala Gly Trp
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Thr Leu Asp Met Ile Gly Ala Ser xaa xaa Thr Trp Glu xaa Gln His
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Pro xaa His xaa xaa xaa xaa Tyr His xaa Phe Gln His xaa Tyr xaa
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Pro xaa xaa Phe xaa xaa Met Thr Ile xaa Lys Val xaa xaa Gln Asp
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xaa xaa Val xaa xaa xaa xaa Arg Leu xaa xaa Ile Asp Ala xaa xaa
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Arg Tyr xaa Ser xaa xaa xaa Val xaa Arg Phe Trp xaa Met Lys xaa
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xaa Thr xaa Leu Tyr xaa xaa xaa Leu Pro xaa Tyr xaa xaa Gly xaa
385                 390                 395                 400
xaa xaa Gly Leu xaa Leu Phe xaa xaa Ala His xaa xaa Cys Gly Glu
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xaa Leu Ala Thr Met Phe Ile Val Asn His xaa Ile Glu Gly Val Ser
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xaa xaa xaa xaa Pro xaa Thr xaa xaa Gly xaa Thr Pro Met xaa xaa
    450                 455                 460
Thr xaa Lys xaa xaa xaa xaa xaa xaa Ser xaa xaa xaa xaa xaa xaa
465                 470                 475                 480
xaa xaa xaa xaa xaa xaa xaa xaa xaa Pro xaa xaa Asp Trp Ala xaa
                485                 490                 495
Val Gln Cys Gln Thr Ser Val Asn Trp xaa xaa Gly Ser Trp Phe Trp
            500                 505                 510
Asn His Phe Ser Gly Gly Leu Asn His Gln Ile Glu His His xaa Phe
        515                 520                 525
Pro xaa xaa xaa His xaa xaa xaa xaa xaa Ile xaa xaa xaa Val xaa
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<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is any or no amino acid
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is any or no amino acid
<220>
<221> Variant
<222> (42)..(42)
<223> xaa in position 42 is Ala or Gly
<220>
<221> Variant
<222> (43)..(43)
<223> xaa in position 43 is Phe or Met
<220>
<221> Variant
<222> (47)..(47)
<223> xaa in position 47 is Phe, Leu or Met
<220>
<221> Variant
<222> (50)..(50)
<223> xaa in position 50 is any amino acid
<220>
<221> Variant
<222> (59)..(59)
<223> xaa in position 59 is Leu or Val
<220>
<221> Variant
<222> (60)..(60)
<223> xaa in position 60 is Ile or Leu
<220>
<221> Variant
<222> (61)..(61)
<223> xaa in position 61 is Asp or Glu

<400> 97
```
Gly xaa Phe Ala Ala Phe xaa Gly Thr Cys Ile Gln His Asp Gly xaa
  1               5                  10                  15
His Gly Ala Phe xaa xaa xaa xaa xaa xaa Asn Lys xaa Ala xaa Gly
            20                  25                  30
Trp Thr Leu Asp Met Ile Gly Ala Ser xaa xaa Thr Trp Glu xaa Gln
        35                  40                  45
His xaa Leu Gly His His Pro Tyr Thr Asn xaa xaa xaa
    50                  55                  60
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<210> 98

<211> 40
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is Lys or Arg
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Phe or Tyr
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Ile or Leu
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Ala or Gly
<220>
<221> Variant
<222> (11)..(12)
<223> xaa in position 11 to 12 is any or no amino acid
<220>
<221> Variant
<222> (14)..(15)
<223> xaa in position 14 to 15 is any amino acid
<220>
<221> Variant
<222> (16)..(17)
<223> xaa in position 16 to 17 is any or no amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Ile or Leu
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ala or Asn
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Phe, Ile or Val
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is Gln, Ser or Thr
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Phe or Val
<220>
<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is Glu or Gly
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is Ala or Val
<220>
<221> Variant
<222> (32)..(32)
<223> xaa in position 32 is any amino acid
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Arg or Ser

<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is any amino acid
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Phe or Tyr
<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is any amino acid

<400> 98
Tyr His xaa xaa Gln His xaa Tyr xaa Pro xaa xaa Phe xaa xaa xaa
 1   5   10   15
xaa Met Thr xaa xaa Lys Val xaa xaa Gln Asp xaa xaa Val xaa xaa
            20   25   30
xaa xaa Arg Leu xaa xaa Ile Asp
        35   40

<210> 99
<211> 59
<212> PRT
<213> Artificial sequence

<220>
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<222> (3)..(3)
<223> xaa in position 3 is Ala or Ile
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Ala, Leu or Val
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Ile or Leu
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is any amino acid
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is Gly or Leu
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Met or Val
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Ala or Gly
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Phe or Met
<220>

<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is any amino acid
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Pro or Val
<220>
<221> Variant
<222> (23)..(24)
<223> xaa in position 23 to 24 is any amino acid
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is Leu or Val
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is any amino acid
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is any amino acid
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is Ile or Met
<220>
<221> Variant
<222> (32)..(32)
<223> xaa in position 32 is Ala or Gly
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Phe or Leu
<220>
<221> Variant
<222> (35)..(35)
<223> xaa in position 35 is Ala or Thr
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Leu or Val
<220>
<221> Variant
<222> (49)..(49)
<223> xaa in position 49 is Ile or Val
<220>
<221> Variant
<222> (56)..(56)
<223> xaa in position 56 is Ala or Gly
<220>
<221> Variant
<222> (57)..(57)
<223> xaa in position 57 is Ser or Thr

<400> 99
Phe Trp xaa Met Lys xaa xaa Thr xaa xaa Tyr xaa xaa xaa Leu Pro
1 5 10 15
xaa Tyr xaa xaa Gly xaa xaa xaa Gly xaa xaa Leu Phe xaa xaa xaa
20 25 30
His xaa xaa Cys Gly Glu xaa Leu Ala Thr Met Phe Ile Val Asn His
35 40 45
xaa Ile Glu Gly Val Ser Tyr xaa xaa Lys Asp
50 55

<210> 100

<211> 45
<212> PRT
<213> Artificial sequence

<220>
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<222> (3)..(3)
<223> xaa in position 3 is Ile or Val
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Asp or Lys
<220>
<221> Variant
<222> (7)..(9)
<223> xaa in position 7 to 9 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Asp or Gly
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ile or Val
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Ala or Gly
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Ala or Leu
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Lys or Arg
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Asp or Glu
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Ala or Cys
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is Ile or Val
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is any amino acid
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Ser or Thr
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Ile, Pro or Val
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is any amino acid

<220>
<221> Variant
<222> (36)..(36)
<223> xaa in position 36 is any amino acid
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Pro or Ser
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is any amino acid
<220>
<221> Variant
<222> (41)..(41)
<223> xaa in position 41 is Leu or Met
<220>
<221> Variant
<222> (42)..(42)
<223> xaa in position 42 is Glu or Arg
<220>
<221> Variant
<222> (45)..(45)
<223> xaa in position 45 is Lys or Arg

<400> 100
Tyr Asp xaa Thr xaa Phe xaa xaa xaa His Pro Gly Gly xaa xaa Ile
1 5 10 15
xaa Leu xaa xaa Gly xaa xaa xaa Thr xaa Leu xaa Glu xaa Tyr His
20 25 30
xaa xaa Gly xaa xaa Asp xaa Val xaa xaa Lys Tyr xaa
35 40 45

<210> 101
<211> 24
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(6)
<223> xaa in position 2 to 6 is any or no amino acid
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Ser or Thr
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Phe or Tyr
<220>
<221> Variant
<222> (19)..(20)
<223> xaa in position 19 to 20 is Leu or Met
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Leu or Met

<400> 101
Asp xaa xaa xaa xaa xaa Gln Glu Ser Asp Pro Asp Val Phe Ser xaa
1 5 10 15
xaa Pro xaa xaa Arg xaa His Pro
20

<210> 102

<211> 27
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Asp or Glu
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is any amino acid
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Leu or Met
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Lys or Arg
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Glu or Arg
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Ala or Val
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Lys or Arg
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (20)..(21)
<223> xaa in position 20 to 21 is any amino acid
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Gln or Arg
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Ala or Gly
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid

<400> 102
Ser xaa Phe Tyr xaa Val xaa xaa xaa Arg Val xaa xaa xaa Leu xaa
 1   5   10   15
Glu xaa Gly xaa xaa xaa Arg xaa xaa xaa Glu
            20   25

<210> 103
<211> 28
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is any or no amino acid
<220>
<221> Variant
<222> (6)..(10)
<223> xaa in position 6 to 10 is any amino acid
<220>
<221> Variant
<222> (11)..(12)
<223> xaa in position 11 to 12 is any or no amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is any amino acid
<220>
<221> Variant
<222> (17)..(18)
<223> xaa in position 17 to 18 is any amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Asp or Thr
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ala or Gly
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is any amino acid
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is Ala or Thr
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is any amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Glu or Gly

<400> 103
Gly xaa xaa xaa Glu xaa xaa xaa xaa xaa xaa xaa Val Arg xaa His
 1   5   10   15
xaa xaa Pro xaa Asp xaa Trp xaa xaa xaa His xaa
            20   25

<210> 104
<211> 28
<212> PRT
<213> Artificial sequence

<220>
<221> Variant

<222> (5)..(5)
<223> xaa in position 5 is any amino acid
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is any or no amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is any or no amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is Phe or Trp
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Ser, Thr or Val
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Ile or Leu
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Glu or Ser
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Lys or Arg
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is any amino acid
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is any amino acid
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Ala or Glu
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Asp, Glu or Lys

<400> 104
Val Pro Tyr Gln xaa Glu xaa Ser xaa Leu xaa xaa Ala Tyr xaa Lys
 1   5   10   15
Met xaa xaa His Leu xaa xaa Leu Gly xaa xaa xaa
            20   25

<210> 105
<211> 15
<212> PRT
<213> Artificial sequence

<220>
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<222> (4)..(4)
<223> xaa in position 4 is any amino acid
<220>

<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Cys or Ser
<220>
<221> Variant
<222> (7)..(8)
<223> xaa in position 7 to 8 is any amino acid
<220>
<221> Variant
<222> (10)..(12)
<223> xaa in position 10 to 12 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is any or no amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ile or Pro

<400> 105
Gly Phe Trp xaa xaa Leu xaa xaa Met xaa xaa xaa xaa Asp xaa
  1   5   10   15

<210> 106
<211> 29
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(7)
<223> xaa in position 2 to 7 is any amino acid
<220>
<221> Variant
<222> (8)..(12)
<223> xaa in position 8 to 12 is any or no amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Asp or Val
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Glu, Asn or Gln
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Gln or Arg
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Glu or Lys
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Ala or Glu
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Ala, Leu or Val
<220>
<221> Variant

<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Ala, Glu or Ser
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Ala, Glu or Asn

<400> 106
Val xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa Thr Pro
 1   5   10   15
Met xaa xaa Thr xaa xaa xaa xaa xaa xaa xaa xaa Ser
            20   25

<210> 107
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Glu or Asn
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is Cys or Val
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Ala or Gly
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Pro or Val
<220>
<221> Variant
<222> (9)..(10)
<223> xaa in position 9 to 10 is any amino acid

<400> 107
Ala xaa xaa Arg Tyr xaa Ser xaa xaa xaa Val
 1   5   10

<210> 108
<211> 8
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Ala or Ile
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Phe, Ile or Leu
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Phe or Leu

<400> 108

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Trp xaa Lys Ala xaa xaa Leu Leu
  1               5

<210> 109
<211> 903
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(903)

<400> 109
atg agc gcc tcc ggt gcg ctg ctg ccc gcg atc gcg ttc gcc gcg tac        48
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1               5                  10                  15
gcg tac gcg acg tac gcc tac gcc ttt gag tgg tcg cac gcg aat ggc        96
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
atc gac aac gtc gac gcg cgc gag tgg atc ggt gcg ctg tcg ttg agg       144
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
ctc ccg gcg atc gcg acg acg atg tac ctg ttg ttc tgc ctg gtc gga       192
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
    50                  55                  60
ccg agg ttg atg gcg aag cgc gag gcg ttc gac ccg aag ggg ttc atg       240
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
ctg gcg tac aat gcg tat cag acg gcg ttc aac gtc gtc gtg ctc ggg       288
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
atg ttc gcg cga gag atc tcg ggg ctg ggg cag ccc gtg tgg ggg tca       336
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
acc atg ccg tgg agc gat aga aaa tcg ttt aag atc ctc ctc ggg gtg       384
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
tgg ttg cac tac aac aac aaa tat ttg gag cta ttg gac act gtg ttc       432
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
atg gtt gcg cgc aag aag acg aag cag ttg agc ttc ttg cac gtt tat       480
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
cat cac gcc ctg ttg atc tgg gcg tgg tgg ttg gtg tgt cac ttg atg       528
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
gcc acg aac gat tgt atc gat gcc tac ttc ggc gcg gcg tgc aac tcg       576
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
ttc att cac atc gtg atg tac tcg tat tat ctc atg tcg gcg ctc ggc       624
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
att cga tgc ccg tgg aag cga tac atc acc cag gct caa atg ctc caa       672
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
    210                 215                 220
ttc gtc att gtc ttc gcg cac gcc gtg ttc gtg ctg cgt cag aag cac       720
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
tgc ccg gtc acc ctt cct tgg gcg caa atg ttc gtc atg acg aac atg       768
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
ctc gtg ctc ttc ggg aac ttc tac ctc aag gcg tac tcg aac aag tcg       816
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
cgc ggc gac ggc gcg agt tcc gtg aaa cca gcc gag acc acg cgc gcg       864
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
ccc agc gtg cga cgc acg cga tct cga aaa att gac taa                   903
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Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300

<210> 110
<211> 300
<212> PRT
<213> Ostreococcus tauri

<400> 110
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1               5                  10                  15
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
    50                  55                  60
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
    210                 215                 220
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300

<210> 111
<211> 834
<212> DNA
<213> Pavlova sp

<220>
<221> CDS
<222> (1)..(834)

<400> 111
atg atg ttg gcc gca ggc tat ctt cta gtg ctc tcg gcc gct cgc cag      48
Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gln
1               5                  10                  15
agc ttc cag cag gac att gac aac ccc aac ggg gcc tac tcg acc tcg      96
Ser Phe Gln Gln Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
            20                  25                  30
tgg act ggc ctg ccc att gtg atg tct gtg gtc tat ctc agc ggt gtg     144
Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
        35                  40                  45
ttt ggg ctc aca aag tac ttc gag aac cgg aag ccc atg acg ggg ctg     192
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Phe Gly Leu Thr Lys Tyr Phe Glu Asn Arg Lys Pro Met Thr Gly Leu
    50                  55                  60
aag gac tac atg ttc act tac aat ctc tac cag gtg atc atc aac gtg      240
Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gln Val Ile Ile Asn Val
65                  70                  75                  80
tgg tgc gtg gtg gcc ttt ctc ctg gag gtg cgg cgt gcg ggc atg tca      288
Trp Cys Val Val Ala Phe Leu Leu Glu Val Arg Arg Ala Gly Met Ser
                85                  90                  95
ctc atc ggc aat aag gtg gac ctt ggg ccc aac tcc ttc agg ctc ggc      336
Leu Ile Gly Asn Lys Val Asp Leu Gly Pro Asn Ser Phe Arg Leu Gly
            100                 105                 110
ttc gtc acg tgg gtg cac tac aac aac aag tac gtg gag ctc ctc gac      384
Phe Val Thr Trp Val His Tyr Asn Asn Lys Tyr Val Glu Leu Leu Asp
        115                 120                 125
acc cta tgg atg gtg ctg cgc aag aag acg cag cag gtc tcc ttc ctc      432
Thr Leu Trp Met Val Leu Arg Lys Lys Thr Gln Gln Val Ser Phe Leu
    130                 135                 140
cac gtc tat cat cac gtg ctt ctg atg tgg gcc tgg ttc gtt gtc gtc      480
His Val Tyr His His Val Leu Leu Met Trp Ala Trp Phe Val Val Val
145                 150                 155                 160
aag ctc ggc aat ggt ggt gac gca tat ttt ggc ggt ctc atg aac tcg      528
Lys Leu Gly Asn Gly Gly Asp Ala Tyr Phe Gly Gly Leu Met Asn Ser
                165                 170                 175
atc atc cac gtg atg atg tat tcc tac tac acc atg gcg ctc ctg ggc      576
Ile Ile His Val Met Met Tyr Ser Tyr Tyr Thr Met Ala Leu Leu Gly
            180                 185                 190
tgg tca tgc ccc tgg aag cgc tac ctc acg cag gca cag ctc gtg cag      624
Trp Ser Cys Pro Trp Lys Arg Tyr Leu Thr Gln Ala Gln Leu Val Gln
        195                 200                 205
ttt tgc atc tgc ctc gcc cac tcc aca tgg gcg gca gta acg ggt gcc      672
Phe Cys Ile Cys Leu Ala His Ser Thr Trp Ala Ala Val Thr Gly Ala
    210                 215                 220
tac ccg tgg cga att tgc ttg gtg gag gtg tgg gtg atg gtg tcc atg      720
Tyr Pro Trp Arg Ile Cys Leu Val Glu Val Trp Val Met Val Ser Met
225                 230                 235                 240
ctg gtg ctc ttc aca cgc ttc tac cgc cag gcc tat gcc aag gag gcg      768
Leu Val Leu Phe Thr Arg Phe Tyr Arg Gln Ala Tyr Ala Lys Glu Ala
                245                 250                 255
aag gcc aag gag gcg aaa aag ctc gca cag gag gca tca cag gcc aag      816
Lys Ala Lys Glu Ala Lys Lys Leu Ala Gln Glu Ala Ser Gln Ala Lys
            260                 265                 270
gcg gtc aag gcg gag taa                                             834
Ala Val Lys Ala Glu
        275
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<210> 112
<211> 277
<212> PRT
<213> Pavlova sp

<400> 112

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Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gln
1               5                   10                  15
Ser Phe Gln Gln Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
            20                  25                  30
Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
        35                  40                  45
Phe Gly Leu Thr Lys Tyr Phe Glu Asn Arg Lys Pro Met Thr Gly Leu
    50                  55                  60
Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gln Val Ile Ile Asn Val
65                  70                  75                  80
Trp Cys Val Val Ala Phe Leu Leu Glu Val Arg Arg Ala Gly Met Ser
                85                  90                  95
Leu Ile Gly Asn Lys Val Asp Leu Gly Pro Asn Ser Phe Arg Leu Gly
            100                 105                 110
Phe Val Thr Trp Val His Tyr Asn Asn Lys Tyr Val Glu Leu Leu Asp
        115                 120                 125
Thr Leu Trp Met Val Leu Arg Lys Lys Thr Gln Gln Val Ser Phe Leu
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    130                 135                 140
His Val Tyr His His Val Leu Leu Met Trp Ala Trp Phe Val Val Val
145                 150                 155                 160
Lys Leu Gly Asn Gly Gly Asp Ala Tyr Phe Gly Gly Leu Met Asn Ser
                165                 170                 175
Ile Ile His Val Met Met Tyr Ser Tyr Tyr Thr Met Ala Leu Leu Gly
            180                 185                 190
Trp Ser Cys Pro Trp Lys Arg Tyr Leu Thr Gln Ala Gln Leu Val Gln
        195                 200                 205
Phe Cys Ile Cys Leu Ala His Ser Thr Trp Ala Ala Val Thr Gly Ala
    210                 215                 220
Tyr Pro Trp Arg Ile Cys Leu Val Glu Val Trp Val Met Val Ser Met
225                 230                 235                 240
Leu Val Leu Phe Thr Arg Phe Tyr Arg Gln Ala Tyr Ala Lys Glu Ala
                245                 250                 255
Lys Ala Lys Glu Ala Lys Lys Leu Ala Gln Glu Ala Ser Gln Ala Lys
            260                 265                 270
Ala Val Lys Ala Glu
        275

<210> 113
<211> 1077
<212> DNA
<213> Thalassiosira pseudonana

<220>
<221> CDS
<222> (1)..(1077)

<400> 113
atg tgc tca tca ccg ccg tca caa tcc aaa aca aca tcc ctc cta gca        48
Met Cys Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala
1               5                   10                  15
cgg tac acc acc gcc gcc ctc ctc ctc ctc acc ctc aca aca tgg tgc        96
Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Trp Cys
            20                  25                  30
cac ttc gcc ttc cca gcc gcc acc gcc aca ccc ggc ctc acc gcc gaa       144
His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu
        35                  40                  45
atg cac tcc tac aaa gtc cca ctc ggt ctc acc gta ttc tac ctg ctg       192
Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu
    50                  55                  60
agt cta ccg tca cta aag tac gtt acg gac aac tac ctt gcc aaa aag       240
Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys
65                  70                  75                  80
tat gat atg aag tca ctc cta acg gaa tca atg gtg ttg tac aat gtg       288
Tyr Asp Met Lys Ser Leu Leu Thr Glu Ser Met Val Leu Tyr Asn Val
                85                  90                  95
gcg caa gtg ctg ctc aat ggg tgg acg gtg tat gcg att gtg gat gcg       336
Ala Gln Val Leu Leu Asn Gly Trp Thr Val Tyr Ala Ile Val Asp Ala
            100                 105                 110
gtg atg aat aga gac cat ccg ttt att gga agt aga agt ttg gtt ggg       384
Val Met Asn Arg Asp His Pro Phe Ile Gly Ser Arg Ser Leu Val Gly
        115                 120                 125
gcg gcg ttg cat agt ggg agc tcg tat gcg gtg tgg gtt cat tat tgt       432
Ala Ala Leu His Ser Gly Ser Ser Tyr Ala Val Trp Val His Tyr Cys
    130                 135                 140
gat aag tat ttg gag ttc ttt gat acg tat ttt atg gtg ttg agg ggg       480
Asp Lys Tyr Leu Glu Phe Phe Asp Thr Tyr Phe Met Val Leu Arg Gly
145                 150                 155                 160
aaa atg gac cag gtc tcc ttc ctc cac atc tac cac cac acg acc ata       528
Lys Met Asp Gln Val Ser Phe Leu His Ile Tyr His His Thr Thr Ile
                165                 170                 175
gcg tgg gca tgg tgg atc gcc ctc cgc ttc tcc ccc ggt gga gac att       576
Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly Gly Asp Ile
            180                 185                 190
tac ttc ggg gca ctc ctc aac tcc atc atc cac gtc ctc atg tat tcc       624
Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu Met Tyr Ser
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          195                 200                 205
tac tac gcc ctt gcc cta ctc aag gtc agt tgt cca tgg aaa cga tac        672
Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp Lys Arg Tyr
    210                 215                 220
ctg act caa gct caa tta ttg caa ttc aca agt gtg gtg gtt tat acg        720
Leu Thr Gln Ala Gln Leu Leu Gln Phe Thr Ser Val Val Val Tyr Thr
225                 230                 235                 240
ggg tgt acg ggt tat act cat tac tat cat acg aag cat gga gcg gat        768
Gly Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His Gly Ala Asp
                245                 250                 255
gag aca cag cct agt tta gga acg tat tat ttc tgt tgt gga gtg cag        816
Glu Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln
            260                 265                 270
gtg ttt gag atg gtt agt ttg ttt gta ctc ttt tcc atc ttt tat aaa        864
Val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
        275                 280                 285
cga tcc tat tcg aag aag aac aag tca gga gga aag gat agc aag aag        912
Arg Ser Tyr Ser Lys Lys Asn Lys Ser Gly Gly Lys Asp Ser Lys Lys
    290                 295                 300
aat gat gat ggg aat aat gag gat caa tgt cac aag gct atg aag gat        960
Asn Asp Asp Gly Asn Asn Glu Asp Gln Cys His Lys Ala Met Lys Asp
305                 310                 315                 320
ata tcg gag ggt gcg aag gag gtt gtg ggg cat gca gcg aag gat gct       1008
Ile Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala
                325                 330                 335
gga aag ttg gtg gct acg gcg agt aag gct gta aag agg aag gga act       1056
Gly Lys Leu Val Ala Thr Ala Ser Lys Ala Val Lys Arg Lys Gly Thr
            340                 345                 350
cgt gtt act ggt gcc atg tag                                          1077
Arg Val Thr Gly Ala Met
        355

<210> 114
<211> 358
<212> PRT
<213> Thalassiosira pseudonana

<400> 114
Met Cys Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala
1               5                  10                  15
Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Trp Cys
            20                  25                  30
His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu
        35                  40                  45
Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu
    50                  55                  60
Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys
65                  70                  75                  80
Tyr Asp Met Lys Ser Leu Leu Thr Glu Ser Met Val Leu Tyr Asn Val
                85                  90                  95
Ala Gln Val Leu Leu Asn Gly Trp Thr Val Tyr Ala Ile Val Asp Ala
            100                 105                 110
Val Met Asn Arg Asp His Pro Phe Ile Gly Ser Arg Ser Leu Val Gly
        115                 120                 125
Ala Ala Leu His Ser Gly Ser Ser Tyr Ala Val Trp Val His Tyr Cys
    130                 135                 140
Asp Lys Tyr Leu Glu Phe Phe Asp Thr Tyr Phe Met Val Leu Arg Gly
145                 150                 155                 160
Lys Met Asp Gln Val Ser Phe Leu His Ile Tyr His His Thr Thr Ile
                165                 170                 175
Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly Gly Asp Ile
            180                 185                 190
Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu Met Tyr Ser
        195                 200                 205
Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp Lys Arg Tyr
    210                 215                 220
Leu Thr Gln Ala Gln Leu Leu Gln Phe Thr Ser Val Val Val Tyr Thr
225                 230                 235                 240
```

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Gly Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His Gly Ala Asp
                245                 250                 255
Glu Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln
            260                 265                 270
Val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
        275                 280                 285
Arg Ser Tyr Ser Lys Lys Asn Lys Ser Gly Gly Lys Asp Ser Lys Lys
    290                 295                 300
Asn Asp Asp Gly Asn Asn Glu Asp Gln Cys His Lys Ala Met Lys Asp
305                 310                 315                 320
Ile Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala
                325                 330                 335
Gly Lys Leu Val Ala Thr Ala Ser Lys Ala Val Lys Arg Lys Gly Thr
            340                 345                 350
Arg Val Thr Gly Ala Met
        355
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<210> 115
<211> 903
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(903)

<400> 115

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atg agc gcc tcc ggt gcg ctg ctg ccc gcg atc gcg tcc gcc gcg tac        48
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Ser Ala Ala Tyr
1               5                  10                  15
gcg tac gcg acg tac gcc tac gcc ttt gag tgg tcg cac gcg aat ggc        96
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
atc gac aac gtc gac gcg cgc gag tgg atc ggt gcg ctg tcg ttg agg       144
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
ctc ccg gcg atc gcg acg acg atg tac ctg ttg ttc tgc ctg gtc gga       192
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
    50                  55                  60
ccg agg ttg atg gcg aag cgc gag gcg ttc gac ccg aag ggg ttc atg       240
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
ctg gcg tac aat gcg tat cag acg gcg ttc aac gtc gtc gtg ctc ggg       288
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
atg ttc gcg cga gag atc tcg ggg ctg ggg cag ccc gtg tgg ggg tca       336
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
acc atg ccg tgg agc gat aga aaa tcg ttt aag atc ctc ctc ggg gtg       384
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
tgg ttg cac tac aac aac aaa tat ttg gag cta ttg gac act gtg ttc       432
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
atg gtt gcg cgc aag aag acg aag cag ttg agc ttc ttg cac gtt tat       480
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
cat cac gcc ctg ttg atc tgg gcg tgg tgg ttg gtg tgt cac ttg atg       528
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
gcc acg aac gat tgt atc gat gcc tac ttc ggc gcg gcg tgc aac tcg       576
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
ttc att cac atc gtg atg tac tcg tat tat ctc atg tcg gcg ctc ggc       624
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
att cga tgc ccg tgg aag cga tac atc acc cag gct caa atg ctc caa       672
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
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```
    210                 215                 220
ttc gtc att gtc ttc gcg cac gcc gtg ttc gtg ctg cgt cag aag cac        720
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
tgc ccg gtc acc ctt cct tgg gcg caa atg ttc gtc atg acg aac atg        768
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
ctc gtg ctc ttc ggg aac ttc tac ctc aag gcg tac tcg aac aag tcg        816
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
cgc ggc gac ggc gcg agt tcc gtg aaa cca gcc gag acc acg cgc gcg        864
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
ccc agc gtg cga cgc acg cga tct cga aaa att gac taa                  903
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300
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<210> 116
<211> 300
<212> PRT
<213> Ostreococcus tauri

<400> 116

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Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Ser Ala Ala Tyr
1               5                  10                  15
Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
            20                  25                  30
Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
        35                  40                  45
Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
    50                  55                  60
Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65                  70                  75                  80
Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
                85                  90                  95
Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
            100                 105                 110
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
        115                 120                 125
Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
    130                 135                 140
Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145                 150                 155                 160
His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
                165                 170                 175
Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
            180                 185                 190
Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
        195                 200                 205
Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
    210                 215                 220
Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
225                 230                 235                 240
Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
                245                 250                 255
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
            260                 265                 270
Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
        275                 280                 285
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
    290                 295                 300
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<210> 117
<211> 339
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (3)..(29)
<223> xaa in position 3 to 29 is any amino acid
<220>
<221> Variant
<222> (30)..(43)
<223> xaa in position 30 to 43 is any or no amino acid
<220>
<221> Variant
<222> (45)..(50)
<223> xaa in position 45 to 50 is any amino acid
<220>
<221> Variant
<222> (53)..(73)
<223> xaa in position 53 to 73 is any amino acid
<220>
<221> Variant
<222> (74)..(79)
<223> xaa in position 74 to 79 is any or no amino acid
<220>
<221> Variant
<222> (81)..(82)
<223> xaa in position 81 to 82 is any amino acid
<220>
<221> Variant
<222> (85)..(86)
<223> xaa in position 85 to 86 is any amino acid
<220>
<221> Variant
<222> (88)..(90)
<223> xaa in position 88 to 90 is any amino acid
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<221> Variant
<222> (92)..(110)
<223> xaa in position 92 to 110 is any amino acid
<220>
<221> Variant
<222> (112)..(127)
<223> xaa in position 112 to 127 is any amino acid
<220>
<221> Variant
<222> (128)..(128)
<223> xaa in position 128 is any or no amino acid
<220>
<221> Variant
<222> (130)..(130)
<223> xaa in position 130 is any amino acid
<220>
<221> Variant
<222> (133)..(134)
<223> xaa in position 133 to 134 is any amino acid
<220>
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<222> (137)..(137)
<223> xaa in position 137 is any amino acid
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<221> Variant
<222> (139)..(140)
<223> xaa in position 139 to 140 is any amino acid
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<221> Variant
<222> (143)..(144)
<223> xaa in position 143 to 144 is any amino acid
<220>
<221> Variant
<222> (147)..(147)
<223> xaa in position 147 is any amino acid

<220>
<221> Variant
<222> (149)..(149)
<223> xaa in position 149 is any amino acid
<220>
<221> Variant
<222> (151)..(152)
<223> xaa in position 151 to 152 is any amino acid
<220>
<221> Variant
<222> (154)..(154)
<223> xaa in position 154 is any amino acid
<220>
<221> Variant
<222> (159)..(159)
<223> xaa in position 159 is any amino acid
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<221> Variant
<222> (163)..(166)
<223> xaa in position 163 to 166 is any amino acid
<220>
<221> Variant
<222> (170)..(179)
<223> xaa in position 170 to 179 is any amino acid
<220>
<221> Variant
<222> (180)..(182)
<223> xaa in position 180 to 182 is any or no amino acid
<220>
<221> Variant
<222> (184)..(184)
<223> xaa in position 184 is any amino acid
<220>
<221> Variant
<222> (188)..(190)
<223> xaa in position 188 to 190 is any amino acid
<220>
<221> Variant
<222> (193)..(193)
<223> xaa in position 193 is any amino acid
<220>
<221> Variant
<222> (196)..(197)
<223> xaa in position 196 to 197 is any amino acid
<220>
<221> Variant
<222> (203)..(206)
<223> xaa in position 203 to 206 is any amino acid
<220>
<221> Variant
<222> (208)..(210)
<223> xaa in position 208 to 210 is any amino acid
<220>
<221> Variant
<222> (217)..(217)
<223> xaa in position 217 is any amino acid
<220>
<221> Variant
<222> (222)..(223)
<223> xaa in position 222 to 223 is any amino acid
<220>
<221> Variant
<222> (226)..(252)
<223> xaa in position 226 to 252 is any amino acid
<220>
<221> Variant
<222> (253)..(267)
<223> xaa in position 253 to 267 is any or no amino acid

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<220>
<221> Variant
<222> (269)..(272)
<223> xaa in position 269 to 272 is any amino acid
<220>
<221> Variant
<222> (276)..(277)
<223> xaa in position 276 to 277 is any amino acid
<220>
<221> Variant
<222> (280)..(282)
<223> xaa in position 280 to 282 is any amino acid
<220>
<221> Variant
<222> (284)..(291)
<223> xaa in position 284 to 291 is any amino acid
<220>
<221> Variant
<222> (292)..(324)
<223> xaa in position 292 to 324 is any or no amino acid
<220>
<221> Variant
<222> (326)..(337)
<223> xaa in position 326 to 337 is any amino acid
<220>
<221> Variant
<222> (338)..(338)
<223> xaa in position 338 is any or no amino acid

<400> 117
Leu Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
  1               5                  10                  15
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
            20                  25                  30
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Pro xaa xaa xaa xaa xaa xaa
        35                  40                  45
xaa xaa Tyr Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
    50                  55                  60
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Met xaa
65                  70                  75                  80
xaa xaa Tyr Asn xaa xaa Gln xaa xaa xaa Asn xaa xaa xaa xaa xaa xaa
                85                  90                  95
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Gly xaa xaa
            100                 105                 110
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
        115                 120                 125
Trp xaa His Tyr xaa xaa Lys Tyr xaa Glu xaa xaa Asp Thr xaa xaa xaa
    130                 135                 140
Met Val xaa Arg xaa Lys xaa xaa Gln xaa Ser Phe Leu His xaa Tyr xaa
145                 150                 155                 160
His His xaa xaa xaa xaa Trp Ala Trp xaa xaa xaa xaa xaa xaa xaa xaa
                165                 170                 175
xaa xaa xaa xaa xaa xaa Asp xaa Tyr Phe Gly xaa xaa xaa Asn Ser xaa
            180                 185                 190
xaa Ile His xaa xaa Met Tyr Ser Tyr Tyr xaa xaa xaa xaa Leu xaa xaa
        195                 200                 205
xaa xaa Cys Pro Trp Lys Arg Tyr xaa Thr Gln Ala Gln xaa xaa Gln xaa
    210                 215                 220
Phe xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
225                 230                 235                 240
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
                245                 250                 255
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Met xaa xaa xaa xaa xaa
            260                 265                 270
Val Leu Phe xaa xaa Phe Tyr xaa xaa xaa Tyr xaa xaa xaa xaa xaa xaa
        275                 280                 285
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
    290                 295                 300
```

```
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
305                 310                 315                 320
xaa xaa xaa xaa Ala xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
                325                 330                 335
xaa xaa Val



<210> 118
<211> 45
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Ala or Gly
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ala or Leu
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Phe or Ile
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is any or no amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any or no amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is any amino acid
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Leu or Met
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ala or Ser
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Ala or Leu
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Arg or Ser
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Ile or Leu
<220>
<221> Variant
<222> (39)..(39)
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<223> xaa in position 39 is Leu or Met
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is Leu or Val
<220>
<221> Variant
<222> (43)..(43)
<223> xaa in position 43 is Cys, Thr or Val
<220>
<221> Variant
<222> (44)..(44)
<223> xaa in position 44 is any amino acid
<220>
<221> Variant
<222> (45)..(45)
<223> xaa in position 45 is Cys or Val

<400> 118
Tyr Phe Gly xaa xaa xaa Asn Ser xaa Ile His xaa Val xaa Met Tyr
 1   5   10   15
Ser Tyr Tyr xaa xaa xaa xaa Leu xaa xaa xaa Cys Pro Trp Lys Arg
            20   25   30
Tyr xaa Thr Gln Ala Gln xaa xaa Gln Phe xaa xaa xaa
        35   40   45

<210> 119
<211> 43
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Leu or Val
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Cys or Asn
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Asp or Asn
<220>
<221> Variant
<222> (9)..(10)
<223> xaa in position 9 to 10 is any or no amino acid
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is any amino acid
<220>
<221> Variant
<222> (13)..(14)
<223> xaa in position 13 to 14 is any or no amino acid
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Phe or Trp
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ala or Leu

<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is any amino acid
<220>
<221> Variant
<222> (25)..(26)
<223> xaa in position 25 to 26 is any amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Leu or Val
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Ile or Val
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Ala, Thr or Val
<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is any amino acid
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Ile or Leu
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is any amino acid

<400> 119
Trp xaa His Tyr xaa xaa Lys Tyr xaa xaa Leu xaa xaa xaa Asp Thr
  1   5   10   15
xaa xaa Met Val xaa Arg xaa Lys xaa xaa Gln xaa Ser Phe Leu His
            20   25   30
xaa Tyr His His xaa xaa xaa xaa Trp Ala Trp
        35   40

<210> 120
<211> 37
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any or no amino acid
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is any or no amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Gly, Ser or Thr
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (13)..(14)
<223> xaa in position 13 to 14 is any amino acid
<220>

<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ala or Ser
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Ala or Ser
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Glu or Lys
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Ala, Asn or Ser
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Lys or Arg
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ala, Gly or Ser
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is any amino acid
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Glu or Gly
<220>
<221> Variant
<222> (25)..(29)
<223> xaa in position 25 to 29 is any amino acid
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Asn, Pro or Gln
<220>
<221> Variant
<222> (31)..(32)
<223> xaa in position 31 to 32 is Ala, Asp or Glu
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Gly, Ser or Thr
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Asn, Gln or Thr
<220>
<221> Variant
<222> (35)..(36)
<223> xaa in position 35 to 36 is any amino acid
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Ala, Asp or Pro

<400> 120
Met xaa xaa Leu xaa Val Leu Phe xaa xaa Phe Tyr xaa xaa xaa Tyr
 1   5   10   15
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa

20 25 30
xaa xaa xaa xaa xaa
35

<210> 121
<211> 19
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant
<222> (4)..(7)
<223> xaa in position 4 to 7 is any or no amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Phe, Leu or Val
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Ala, Leu or Val
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any amino acid
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Thr or Val
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Ala, Ile or Leu
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Phe, Ile or Leu

<400> 121
Lys xaa xaa xaa xaa xaa xaa Met xaa xaa Tyr Asn xaa xaa Gln xaa
1 5 10 15
xaa xaa Asn

<210> 122
<211> 879
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(879)

<400> 122
atg tct gga ttg agg gct cct aac ttc ttg cat agg ttc tgg acc aag 48
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1 5 10 15
tgg gat tac gct atc tct aag gtg gtg ttc act tgc gct gat tct ttc 96
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe

```
                20                  25                  30
cag tgg gat atc gga cct gtt tct tct tct acc gct cat ttg cct gct  144
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
att gag tct cct act cct ttg gtg acc tct ttg ctc ttc tac ttg gtg  192
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
act gtg ttc ttg tgg tac gga aga ttg acc aga tcc tcc gat aag aag  240
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
atc aga gag cct acc tgg ttg agg aga ttc atc atc tgc cac aac gct  288
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
ttc ttg att gtg ctc tcc ttg tac atg tgt ttg gga tgc gtt gct caa  336
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
gct tac caa aac gga tac acc ttg tgg gga aac gag ttc aag gct act  384
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
gag acc caa ttg gct ctc tac atc tac atc ttc tac gtg tcc aag atc  432
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
tac gag ttc gtg gat acc tac atc atg ctc ctc aag aac aac ctc agg  480
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
caa gtg tct ttc ttg cac atc tac cac cac tct acc atc tct ttc atc  528
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
tgg tgg atc atc gct aga aga gca cct gga gga gat gct tat ttc tcc  576
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
gct gct ctc aac tct tgg gtt cat gtg tgc atg tac act tac tac ctc  624
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
ctc tct acc ttg att gga aag gaa gat cct aag agg tct aac tac ctc  672
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
tgg tgg gga agg cat ttg acc caa atg caa atg ctc cag ttc ttc ttc  720
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
aac gtg ctc caa gct ctt tat tgc gct tcc ttc tcc act tac cct aag  768
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
ttc ctc tcc aag atc ttg ctc gtg tac atg atg tct ttg ctc gga ctt  816
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
ttc gga cac ttc tac tac tct aag cac atc gct gct gct aag ttg caa  864
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
aag aag cag cag tga                                             879
Lys Lys Gln Gln
    290
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<210> 123
<211> 292
<212> PRT
<213> Ostreococcus tauri

<400> 123

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Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
```

```
65                  70                  75                  80
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
Lys Lys Gln Gln
    290
```

<210> 124
<211> 1047
<212> DNA
<213> Marchantia polymorpha

<220>
<221> CDS
<222> (1)..(1047)

<400> 124

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atg gcg acg aag agc ggg agc gga ttg ctc gag tgg ata gca gta gcg        48
Met Ala Thr Lys Ser Gly Ser Gly Leu Leu Glu Trp Ile Ala Val Ala
1               5                   10                  15
gcg aag atg aag caa gct cgg agc agc ccc gag ggt gag atc gtg ggt        96
Ala Lys Met Lys Gln Ala Arg Ser Ser Pro Glu Gly Glu Ile Val Gly
            20                  25                  30
ggg aat agg atg ggc tct gga aac gga gct gag tgg acc acg agt ctg       144
Gly Asn Arg Met Gly Ser Gly Asn Gly Ala Glu Trp Thr Thr Ser Leu
        35                  40                  45
att cat gca ttt ttg aat gcc acg aat ggg aag agc ggc ggt gct tcg       192
Ile His Ala Phe Leu Asn Ala Thr Asn Gly Lys Ser Gly Gly Ala Ser
    50                  55                  60
aaa gtg agg cct ctc gag gag aga atc ggg gag gcg gtg ttc aga gtt       240
Lys Val Arg Pro Leu Glu Glu Arg Ile Gly Glu Ala Val Phe Arg Val
65                  70                  75                  80
ctt gaa gat gtc gtg ggc gtg gat att agg aag ccg aat cct gtc acg       288
Leu Glu Asp Val Val Gly Val Asp Ile Arg Lys Pro Asn Pro Val Thr
                85                  90                  95
aag gac ctt ccg atg gtc gag agt ccc gtg ccc gtg ttg gcc tgc att       336
Lys Asp Leu Pro Met Val Glu Ser Pro Val Pro Val Leu Ala Cys Ile
            100                 105                 110
tct ctg tac ttg ctc gtg gtg tgg ctt tgg tct tct cac att aag gcg       384
Ser Leu Tyr Leu Leu Val Val Trp Leu Trp Ser Ser His Ile Lys Ala
        115                 120                 125
tct ggc caa aag ccc agg aag gag gac ccg ctc gcc ctg cgt tgc ctt       432
Ser Gly Gln Lys Pro Arg Lys Glu Asp Pro Leu Ala Leu Arg Cys Leu
    130                 135                 140
gtg att gcc cac aat ctg ttc ctg tgt tgc ttg agc ttg ttc atg tgc       480
```

```
Val Ile Ala His Asn Leu Phe Leu Cys Cys Leu Ser Leu Phe Met Cys
145                 150                 155                 160
gtc ggt ctc att gcc gca gct cga cat tac ggg tat agt gta tgg ggg        528
Val Gly Leu Ile Ala Ala Ala Arg His Tyr Gly Tyr Ser Val Trp Gly
                165                 170                 175
aac tac tac aga gaa aga gaa ccc gca atg aat ttg ctc att tac gtg        576
Asn Tyr Tyr Arg Glu Arg Glu Pro Ala Met Asn Leu Leu Ile Tyr Val
            180                 185                 190
ttc tac atg tcg aag ctg tac gaa ttt atg gac acg gcc att atg tta        624
Phe Tyr Met Ser Lys Leu Tyr Glu Phe Met Asp Thr Ala Ile Met Leu
        195                 200                 205
ttc aga aga aat ctg cga caa gtc acg tac ttg cat gta tat cac cac        672
Phe Arg Arg Asn Leu Arg Gln Val Thr Tyr Leu His Val Tyr His His
    210                 215                 220
gca agc atc gca atg att tgg tgg ata att tgc tat cgg ttt cca gga        720
Ala Ser Ile Ala Met Ile Trp Trp Ile Ile Cys Tyr Arg Phe Pro Gly
225                 230                 235                 240
gct gat tcg tat ttc tcc gca gca ttc aat tcc tgt atc cat gta gcg        768
Ala Asp Ser Tyr Phe Ser Ala Ala Phe Asn Ser Cys Ile His Val Ala
                245                 250                 255
atg tac ctg tat tat cta ctc gcg gca acc gtc gcc aga gac gaa aag        816
Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Val Ala Arg Asp Glu Lys
            260                 265                 270
cgg aga cgc aaa tat ctc ttc tgg gga aag tat ctg acc atc ata caa        864
Arg Arg Arg Lys Tyr Leu Phe Trp Gly Lys Tyr Leu Thr Ile Ile Gln
        275                 280                 285
atg ctt cag ttt ttg tcc ttc att ggg cag gcg att tat gca atg tgg        912
Met Leu Gln Phe Leu Ser Phe Ile Gly Gln Ala Ile Tyr Ala Met Trp
    290                 295                 300
aag ttt gaa tac tat ccc aag ggc ttt ggc agg atg ttg ttc ttt tac        960
Lys Phe Glu Tyr Tyr Pro Lys Gly Phe Gly Arg Met Leu Phe Phe Tyr
305                 310                 315                 320
tct gta tca ttg ttg gca ttt ttc ggc aac ttc ttt gtc aaa aag tat       1008
Ser Val Ser Leu Leu Ala Phe Phe Gly Asn Phe Phe Val Lys Lys Tyr
                325                 330                 335
tcg aac gct tca cag cct aag aca gtt aaa gtg gag tga                   1047
Ser Asn Ala Ser Gln Pro Lys Thr Val Lys Val Glu
            340                 345
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<210> 125
<211> 348
<212> PRT
<213> Marchantia polymorpha

<400> 125

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Met Ala Thr Lys Ser Gly Ser Gly Leu Leu Glu Trp Ile Ala Val Ala
1               5                  10                  15
Ala Lys Met Lys Gln Ala Arg Ser Ser Pro Glu Gly Glu Ile Val Gly
            20                  25                  30
Gly Asn Arg Met Gly Ser Gly Asn Gly Ala Glu Trp Thr Thr Ser Leu
        35                  40                  45
Ile His Ala Phe Leu Asn Ala Thr Asn Gly Lys Ser Gly Gly Ala Ser
    50                  55                  60
Lys Val Arg Pro Leu Glu Glu Arg Ile Gly Glu Ala Val Phe Arg Val
65                  70                  75                  80
Leu Glu Asp Val Val Gly Val Asp Ile Arg Lys Pro Asn Pro Val Thr
                85                  90                  95
Lys Asp Leu Pro Met Val Glu Ser Pro Val Pro Val Leu Ala Cys Ile
            100                 105                 110
Ser Leu Tyr Leu Leu Val Val Trp Leu Trp Ser Ser His Ile Lys Ala
        115                 120                 125
Ser Gly Gln Lys Pro Arg Lys Glu Asp Pro Leu Ala Leu Arg Cys Leu
    130                 135                 140
Val Ile Ala His Asn Leu Phe Leu Cys Cys Leu Ser Leu Phe Met Cys
145                 150                 155                 160
Val Gly Leu Ile Ala Ala Ala Arg His Tyr Gly Tyr Ser Val Trp Gly
                165                 170                 175
Asn Tyr Tyr Arg Glu Arg Glu Pro Ala Met Asn Leu Leu Ile Tyr Val
```

```
                180                 185                 190
Phe Tyr Met Ser Lys Leu Tyr Glu Phe Met Asp Thr Ala Ile Met Leu
        195                 200                 205
Phe Arg Arg Asn Leu Arg Gln Val Thr Tyr Leu His Val Tyr His His
    210                 215                 220
Ala Ser Ile Ala Met Ile Trp Trp Ile Ile Cys Tyr Arg Phe Pro Gly
225                 230                 235                 240
Ala Asp Ser Tyr Phe Ser Ala Ala Phe Asn Ser Cys Ile His Val Ala
                245                 250                 255
Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Val Ala Arg Asp Glu Lys
            260                 265                 270
Arg Arg Arg Lys Tyr Leu Phe Trp Gly Lys Tyr Leu Thr Ile Ile Gln
        275                 280                 285
Met Leu Gln Phe Leu Ser Phe Ile Gly Gln Ala Ile Tyr Ala Met Trp
    290                 295                 300
Lys Phe Glu Tyr Tyr Pro Lys Gly Phe Gly Arg Met Leu Phe Phe Tyr
305                 310                 315                 320
Ser Val Ser Leu Leu Ala Phe Phe Gly Asn Phe Phe Val Lys Lys Tyr
                325                 330                 335
Ser Asn Ala Ser Gln Pro Lys Thr Val Lys Val Glu
            340                 345

<210> 126
<211> 831
<212> DNA
<213> Thraustochytrium sp

<220>
<221> CDS
<222> (1)..(831)

<400> 126
atg gat gtc gtc gag cag caa tgg cgc cgc ttc gtg gac gcc gtg gac        48
Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                   10                  15
aac gga atc gtg gag ttc atg gag cat gag gag ccc aac aag ctg aac        96
Asn Gly Ile Val Glu Phe Met Glu His Glu Glu Pro Asn Lys Leu Asn
            20                  25                  30
gag ggc aag ctc tcc acc tcg acc gag gag atg atg gcg ctt atc gtc       144
Glu Gly Lys Leu Ser Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
ggc tac ctg gcg ttc gtg gtc ctc ggg tcc gcc ttc atg aag gcc ttt       192
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
gtc gat aag cct ttc gag ctc aag ttc ctc aag ctc gtg cac aac atc       240
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
ttc ctc acc ggt ctg tcc atg tac atg gcc acc gag tgc gcg cgc cag       288
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
gca tac ctc ggc ggc tac aag ctc ttt ggc aac ccg atg gag aag ggc       336
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
acc gag tcg cac gcc ccg ggc atg gcc aac atc atc tac atc ttc tac       384
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
gtg agc aag ttc ctc gaa ttc ctc gac acc gtc ttc atg atc ctc ggc       432
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
aag aag tgg aag cag ctc agc ttt ctc cac gtc tac cac cac gcg agc       480
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
atc agc ttc atc tgg ggc atc atc gcc cgc ttc gcg ccc ggt ggc gac       528
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
gcc tac ttc tct acc atc ctc aac agc agc gtg cat gtc gtg ctc tac       576
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
```

```
ggc tac tac gcc tcg acc acc ctc ggc tac acc ttc atg cgc ccg ctg        624
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
cgc ccg tac att acc acc att cag ctc acg cag ttc atg gcc atg gtc        672
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
gtc cag tcc gtc tat gac tac tac aac ccc tgc gac tac ccg cag ccc        720
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
ctc gtc aag ctg ctc ttc tgg tac atg ctc acc atg ctc ggc ctc ttc        768
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
ggc aac ttc ttc gtg cag cag tac ctc aag ccc aag gcg ccc aag aag        816
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
cag aag acc atc taa                                                   831
Gln Lys Thr Ile
        275
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<210> 127
<211> 276
<212> PRT
<213> Thraustochytrium sp

<400> 127

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Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                  10                  15
Asn Gly Ile Val Glu Phe Met Glu His Glu Glu Pro Asn Lys Leu Asn
            20                  25                  30
Glu Gly Lys Leu Ser Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
Gln Lys Thr Ile
        275
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<210> 128
<211> 1146
<212> DNA
<213> Leishmania major

<220>

<221> CDS
<222> (1)..(1146)

<400> 128

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atg gtc tct ctg gag cag gcc gaa cag atc gcg gcc gcg atc gag gta        48
Met Val Ser Leu Glu Gln Ala Glu Gln Ile Ala Ala Ala Ile Glu Val
1               5                  10                  15
cct gac tgg gtc ttg aca aag tct gcg gcg ctg gtg tac agc tgc ttc        96
Pro Asp Trp Val Leu Thr Lys Ser Ala Ala Leu Val Tyr Ser Cys Phe
            20                  25                  30
ggc tcc gcc gcc aat gct ttt gaa agc agt atc aag atc aac ttc ccg       144
Gly Ser Ala Ala Asn Ala Phe Glu Ser Ser Ile Lys Ile Asn Phe Pro
        35                  40                  45
gcg cag cat gcg ttc gtg gag gcg tgg atg cgc gcg cgc tcc cac cct       192
Ala Gln His Ala Phe Val Glu Ala Trp Met Arg Ala Arg Ser His Pro
    50                  55                  60
ttt gcg gag cgc ctg ccg tac ctg aat ccg tgg cac gtt atc gcc tcg       240
Phe Ala Glu Arg Leu Pro Tyr Leu Asn Pro Trp His Val Ile Ala Ser
65                  70                  75                  80
ata ctg gcc tac ctc tcc ttg att gtc acc ttg cgc ctg ttg cat cgt       288
Ile Leu Ala Tyr Leu Ser Leu Ile Val Thr Leu Arg Leu Leu His Arg
                85                  90                  95
gta ctc ggt aag ttc tcg tgc cgc act ctc gga ttg gtg cac aac ctc       336
Val Leu Gly Lys Phe Ser Cys Arg Thr Leu Gly Leu Val His Asn Leu
            100                 105                 110
ggt ctc cat ctt ctc tcg ttg tac atg agc ctt ggt ctc atg atc agc       384
Gly Leu His Leu Leu Ser Leu Tyr Met Ser Leu Gly Leu Met Ile Ser
        115                 120                 125
gcg cgc gcc gcg ggg tac tcg ctc tgg aac aac gcg gtc ggc acc tcc       432
Ala Arg Ala Ala Gly Tyr Ser Leu Trp Asn Asn Ala Val Gly Thr Ser
    130                 135                 140
ccg gct gag tgg cgc att gcg aag ctg atc tgg ctc ttc tat gtc tcg       480
Pro Ala Glu Trp Arg Ile Ala Lys Leu Ile Trp Leu Phe Tyr Val Ser
145                 150                 155                 160
aag gtg gtg gaa tgg gtg gac acg gta att atg tta tta aag cag aac       528
Lys Val Val Glu Trp Val Asp Thr Val Ile Met Leu Leu Lys Gln Asn
                165                 170                 175
tac cac cag gtc acc ttc ctg cac gtg tat cac cac acg acg gtt ttt       576
Tyr His Gln Val Thr Phe Leu His Val Tyr His His Thr Thr Val Phe
            180                 185                 190
gtg ctg tgg tgg ctg gcg ttg ctg gtc gct cct ggc ggc gag tcg tac       624
Val Leu Trp Trp Leu Ala Leu Leu Val Ala Pro Gly Gly Glu Ser Tyr
        195                 200                 205
tac agc gcc atg gtg aac tct ggc gtc cac gtt ttc atg tac ggg tac       672
Tyr Ser Ala Met Val Asn Ser Gly Val His Val Phe Met Tyr Gly Tyr
    210                 215                 220
tac ttt ctc acg ctg ctc ttc cca tcc ggc atc gtg cgc gac gtc ttg       720
Tyr Phe Leu Thr Leu Leu Phe Pro Ser Gly Ile Val Arg Asp Val Leu
225                 230                 235                 240
agc aag ttc aag ttt gcc att acg aag ggc cag atg tgg cag ttc gtc       768
Ser Lys Phe Lys Phe Ala Ile Thr Lys Gly Gln Met Trp Gln Phe Val
                245                 250                 255
ttc aac tgc cta cag tcc gcg tac gac ctc gtg tgg gtg ccg cgg gaa       816
Phe Asn Cys Leu Gln Ser Ala Tyr Asp Leu Val Trp Val Pro Arg Glu
            260                 265                 270
gag ctc aag tac agc gcg gag ctg ctg cag atc ctc ttc tgg tac atg       864
Glu Leu Lys Tyr Ser Ala Glu Leu Leu Gln Ile Leu Phe Trp Tyr Met
        275                 280                 285
atc tcc ctc ttg gcg ctc ttt ggc aac ttc ttg gtg aag aac aag aag       912
Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Leu Val Lys Asn Lys Lys
    290                 295                 300
ttc tcg cac cgc cgc tgc gtt gat gcc gcg act gct tcg ggc gcg aag       960
Phe Ser His Arg Arg Cys Val Asp Ala Ala Thr Ala Ser Gly Ala Lys
305                 310                 315                 320
gag gac acg gcg gcg agg tcc cac ggc gac cgc acc cac aga acc cgt      1008
Glu Asp Thr Ala Ala Arg Ser His Gly Asp Arg Thr His Arg Thr Arg
                325                 330                 335
gtg aag gct ggc atg acc aac atg caa ctg gag agg ctg aag aat gag      1056
```

```
Val Lys Ala Gly Met Thr Asn Met Gln Leu Glu Arg Leu Lys Asn Glu
                340                 345                 350
aag tcc acg gag atg aag ctg ctg atg cgc aag aac ggc aac ggc aac        1104
Lys Ser Thr Glu Met Lys Leu Leu Met Arg Lys Asn Gly Asn Gly Asn
        355                 360                 365
gga caa aaa gcg tcg ctc cag gcc atg gca ggc agt cga tga              1146
Gly Gln Lys Ala Ser Leu Gln Ala Met Ala Gly Ser Arg
    370                 375                 380

<210> 129
<211> 381
<212> PRT
<213> Leishmania major

<400> 129
Met Val Ser Leu Glu Gln Ala Glu Gln Ile Ala Ala Ala Ile Glu Val
1               5                  10                  15
Pro Asp Trp Val Leu Thr Lys Ser Ala Ala Leu Val Tyr Ser Cys Phe
            20                  25                  30
Gly Ser Ala Ala Asn Ala Phe Glu Ser Ser Ile Lys Ile Asn Phe Pro
        35                  40                  45
Ala Gln His Ala Phe Val Glu Ala Trp Met Arg Ala Arg Ser His Pro
    50                  55                  60
Phe Ala Glu Arg Leu Pro Tyr Leu Asn Pro Trp His Val Ile Ala Ser
65                  70                  75                  80
Ile Leu Ala Tyr Leu Ser Leu Ile Val Thr Leu Arg Leu Leu His Arg
                85                  90                  95
Val Leu Gly Lys Phe Ser Cys Arg Thr Leu Gly Leu Val His Asn Leu
            100                 105                 110
Gly Leu His Leu Leu Ser Leu Tyr Met Ser Leu Gly Leu Met Ile Ser
        115                 120                 125
Ala Arg Ala Ala Gly Tyr Ser Leu Trp Asn Asn Ala Val Gly Thr Ser
    130                 135                 140
Pro Ala Glu Trp Arg Ile Ala Lys Leu Ile Trp Leu Phe Tyr Val Ser
145                 150                 155                 160
Lys Val Val Glu Trp Val Asp Thr Val Ile Met Leu Leu Lys Gln Asn
                165                 170                 175
Tyr His Gln Val Thr Phe Leu His Val Tyr His His Thr Thr Val Phe
            180                 185                 190
Val Leu Trp Trp Leu Ala Leu Leu Val Ala Pro Gly Gly Glu Ser Tyr
        195                 200                 205
Tyr Ser Ala Met Val Asn Ser Gly Val His Val Phe Met Tyr Gly Tyr
    210                 215                 220
Tyr Phe Leu Thr Leu Leu Phe Pro Ser Gly Ile Val Arg Asp Val Leu
225                 230                 235                 240
Ser Lys Phe Lys Phe Ala Ile Thr Lys Gly Gln Met Trp Gln Phe Val
                245                 250                 255
Phe Asn Cys Leu Gln Ser Ala Tyr Asp Leu Val Trp Val Pro Arg Glu
            260                 265                 270
Glu Leu Lys Tyr Ser Ala Glu Leu Leu Gln Ile Leu Phe Trp Tyr Met
        275                 280                 285
Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Leu Val Lys Asn Lys Lys
    290                 295                 300
Phe Ser His Arg Arg Cys Val Asp Ala Ala Thr Ala Ser Gly Ala Lys
305                 310                 315                 320
Glu Asp Thr Ala Ala Arg Ser His Gly Asp Arg Thr His Arg Thr Arg
                325                 330                 335
Val Lys Ala Gly Met Thr Asn Met Gln Leu Glu Arg Leu Lys Asn Glu
            340                 345                 350
Lys Ser Thr Glu Met Lys Leu Leu Met Arg Lys Asn Gly Asn Gly Asn
        355                 360                 365
Gly Gln Lys Ala Ser Leu Gln Ala Met Ala Gly Ser Arg
    370                 375                 380

<210> 130
<211> 879
<212> DNA
<213> Ostreococcus tauri
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<220>
<221> CDS
<222> (1)..(879)

<400> 130

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atg agt ggc tta cgt gca ccc aac ttt tta cac aga ttc tgg aca aag        48
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
tgg gac tac gcg att tcc aaa gtc gtc ttc acg tgt gcc gac agt ttt        96
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
cag tgg gac atc ggg cca gtg agt tcg agt acg gcg cat tta ccc gcc       144
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
att gaa tcc cct acc cca ctg gtg act agc ctc ttg ttc tac tta gtc       192
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
aca gtt ttc ttg tgg tat ggt cgt tta acc agg agt tca gac aag aaa       240
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
att aga gag cct acg tgg tta aga aga ttc ata ata tgt cat aat gcg       288
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
ttc ttg ata gtc ctc agt ctt tac atg tgc ctt ggt tgt gtg gcc caa       336
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
gcg tat cag aat gga tat act tta tgg ggt aat gaa ttc aag gcc acg       384
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
gaa act cag ctt gct ctc tac att tac att ttt tac gta agt aaa ata       432
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
tac gag ttt gta gat act tac att atg ctt ctc aag aat aac ttg cgg       480
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
caa gta agt ttc cta cac att tat cac cac agc acg att tcc ttt att       528
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
tgg tgg atc att gct cgg agg gct ccg ggt ggt gat gct tac ttc agc       576
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
gcg gcc ttg aac tca tgg gta cac gtg tgc atg tac acc tat tat cta       624
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
tta tca acc ctt att gga aaa gaa gat cct aag cgt tcc aac tac ctt       672
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
tgg tgg ggt cgc cac cta acg caa atg cag atg ctt cag ttt ttc ttc       720
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
aac gta ctt caa gcg ttg tac tgc gct tcg ttc tct acg tat ccc aag       768
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
ttt ttg tcc aaa att ctg ctc gtc tat atg atg agc ctt ctc ggc ttg       816
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
ttt ggg cat ttc tac tat tcc aag cac ata gca gca gct aag ctc cag       864
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
aaa aaa cag cag tga                                                   879
Lys Lys Gln Gln
    290
```

<210> 131
<211> 292
<212> PRT
<213> Ostreococcus tauri

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<400> 131
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
Lys Lys Gln Gln
    290

<210> 132
<211> 873
<212> DNA
<213> Marchantia polymorpha

<220>
<221> CDS
<222> (1)..(873)

<400> 132
atg gag gcg tac gag atg gtg gat agt ttt gtg tcg aag acg gtt ttc      48
Met Glu Ala Tyr Glu Met Val Asp Ser Phe Val Ser Lys Thr Val Phe
1               5                  10                  15
gaa acg ctg cag aga ctg agg ggc gga gtc gtg ttg acg gaa tct gcg      96
Glu Thr Leu Gln Arg Leu Arg Gly Gly Val Val Leu Thr Glu Ser Ala
            20                  25                  30
atc acc aaa ggt ttg cca tgc gtc gat agc ccg acg ccg atc gtt ctt     144
Ile Thr Lys Gly Leu Pro Cys Val Asp Ser Pro Thr Pro Ile Val Leu
        35                  40                  45
ggg ttg tcg tcc tac ttg aca ttc gtg ttt ctc ggg ctc att gtc atc     192
Gly Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gly Leu Ile Val Ile
    50                  55                  60
aag agc ctg gat ctt aag ccc cgc tcc aag gag ccc gcc att ttg aac     240
Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Glu Pro Ala Ile Leu Asn
65                  70                  75                  80
ctg ttt gtg atc ttc cac aac ttc gtc tgc ttc gca ctc agt ctg tac     288
Leu Phe Val Ile Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr
```

```
                    85                  90                  95
atg tgc gtg gga att gtc cgt caa gct atc ctc aac agg tac tct ctg        336
Met Cys Val Gly Ile Val Arg Gln Ala Ile Leu Asn Arg Tyr Ser Leu
            100                 105                 110
tgg ggc aat gcg tac aat ccc aaa gaa gtt caa atg ggc cac ctg ctc        384
Trp Gly Asn Ala Tyr Asn Pro Lys Glu Val Gln Met Gly His Leu Leu
        115                 120                 125
tac att ttc tac atg tca aag tac atc gag ttt atg gac acg gtc att        432
Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Glu Phe Met Asp Thr Val Ile
    130                 135                 140
atg att ttg aag cgc aac acg cgc cag atc act gtg ttg cat gtg tac        480
Met Ile Leu Lys Arg Asn Thr Arg Gln Ile Thr Val Leu His Val Tyr
145                 150                 155                 160
cac cac gca tcc atc tcc ttc atc tgg tgg atc atc gcc tac cat gct        528
His His Ala Ser Ile Ser Phe Ile Trp Trp Ile Ile Ala Tyr His Ala
                165                 170                 175
cct ggc ggt gaa gct tat ttc tct gcc gca ttg aac tcc gga gta cat        576
Pro Gly Gly Glu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
            180                 185                 190
gtg ctc atg tac ctc tac tac ctt ttg gca gca act ctg gga aag aac        624
Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gly Lys Asn
        195                 200                 205
gag aaa gct cgc cgc aag tac cta tgg tgg gga aaa tac ttg aca cag        672
Glu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr Gln
    210                 215                 220
ctg cag atg ttc cag ttt gtc ctt aac atg att cag gct tac tac gat        720
Leu Gln Met Phe Gln Phe Val Leu Asn Met Ile Gln Ala Tyr Tyr Asp
225                 230                 235                 240
att aag aac aac tcg cct tac cca caa ttt ttg atc cag att ttg ttc        768
Ile Lys Asn Asn Ser Pro Tyr Pro Gln Phe Leu Ile Gln Ile Leu Phe
                245                 250                 255
tac tac atg atc tcg ctt tta gcg cta ttt gga aac ttt tac gtt cac        816
Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val His
            260                 265                 270
aaa tac gta tca gcg ccc gca aaa cct gcg aag atc aag agc aaa aag        864
Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
        275                 280                 285
gca gaa taa                                                           873
Ala Glu
    290
```

<210> 133
<211> 290
<212> PRT
<213> Marchantia polymorpha

<400> 133

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Met Glu Ala Tyr Glu Met Val Asp Ser Phe Val Ser Lys Thr Val Phe
1               5                  10                  15
Glu Thr Leu Gln Arg Leu Arg Gly Gly Val Val Leu Thr Glu Ser Ala
            20                  25                  30
Ile Thr Lys Gly Leu Pro Cys Val Asp Ser Pro Thr Pro Ile Val Leu
        35                  40                  45
Gly Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gly Leu Ile Val Ile
    50                  55                  60
Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Glu Pro Ala Ile Leu Asn
65                  70                  75                  80
Leu Phe Val Ile Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr
                85                  90                  95
Met Cys Val Gly Ile Val Arg Gln Ala Ile Leu Asn Arg Tyr Ser Leu
            100                 105                 110
Trp Gly Asn Ala Tyr Asn Pro Lys Glu Val Gln Met Gly His Leu Leu
        115                 120                 125
Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Glu Phe Met Asp Thr Val Ile
    130                 135                 140
Met Ile Leu Lys Arg Asn Thr Arg Gln Ile Thr Val Leu His Val Tyr
145                 150                 155                 160
His His Ala Ser Ile Ser Phe Ile Trp Trp Ile Ile Ala Tyr His Ala
```

```
                    165                 170                 175
Pro Gly Gly Glu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
                180                 185                 190
Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gly Lys Asn
            195                 200                 205
Glu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr Gln
    210                 215                 220
Leu Gln Met Phe Gln Phe Val Leu Asn Met Ile Gln Ala Tyr Tyr Asp
225                 230                 235                 240
Ile Lys Asn Asn Ser Pro Tyr Pro Gln Phe Leu Ile Gln Ile Leu Phe
                245                 250                 255
Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val His
            260                 265                 270
Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
        275                 280                 285
Ala Glu
    290

<210> 134
<211> 873
<212> DNA
<213> Physcomitrella patens

<220>
<221> CDS
<222> (1)..(873)

<400> 134
atg gag gtc gtg gag aga ttc tac ggt gag ttg gat ggg aag gtc tcg      48
Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
1                   5                  10                  15
cag ggc gtg aat gca ttg ctg ggt agt ttt ggg gtg gag ttg acg gat      96
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30
acg ccc act acc aaa ggc ttg ccc ctc gtt gac agt ccc aca ccc atc     144
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45
gtc ctc ggt gtt tct gta tac ttg act att gtc att gga ggg ctt ttg     192
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60
tgg ata aag gcc agg gat ctg aaa ccg cgc gcc tcg gag cca ttt ttg     240
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80
ctc caa gct ttg gtg ctt gtg cac aac ctg ttc tgt ttt gcg ctc agt     288
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95
ctg tat atg tgc gtg ggc atc gct tat cag gct att acc tgg cgg tac     336
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110
tct ctc tgg ggc aat gca tac aat cct aaa cat aaa gag atg gcg att     384
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125
ctg gta tac ttg ttc tac atg tct aag tac gtg gaa ttc atg gat acc     432
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140
gtt atc atg ata ctg aag cgc agc acc agg caa ata agc ttc ctc cac     480
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160
gtt tat cat cat tct tca att tcc ctc att tgg tgg gct att gct cat     528
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175
cac gct cct ggc ggt gaa gca tat tgg tct gcg gct ctg aac tca gga     576
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190
gtg cat gtt ctc atg tat gcg tat tac ttc ttg gct gcc tgc ctt cga     624
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205
agt agc cca aag tta aaa aat aag tac ctt ttt tgg ggc agg tac ttg     672
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Ser  Ser  Pro  Lys  Leu  Lys  Asn  Lys  Tyr  Leu  Phe  Trp  Gly  Arg  Tyr  Leu
     210                       215                       220
aca  caa  ttc  caa  atg  ttc  cag  ttt  atg  ctg  aac  tta  gtg  cag  gct  tac        720
Thr  Gln  Phe  Gln  Met  Phe  Gln  Phe  Met  Leu  Asn  Leu  Val  Gln  Ala  Tyr
225                       230                       235                       240
tac  gac  atg  aaa  acg  aat  gcg  cca  tat  cca  caa  tgg  ctg  atc  aag  att        768
Tyr  Asp  Met  Lys  Thr  Asn  Ala  Pro  Tyr  Pro  Gln  Trp  Leu  Ile  Lys  Ile
                  245                       250                       255
ttg  ttc  tac  tac  atg  atc  tcg  ttg  ctg  ttt  ctt  ttc  ggc  aat  ttt  tac        816
Leu  Phe  Tyr  Tyr  Met  Ile  Ser  Leu  Leu  Phe  Leu  Phe  Gly  Asn  Phe  Tyr
            260                       265                       270
gta  caa  aaa  tac  atc  aaa  ccc  tct  gac  gga  aag  caa  aag  gga  gct  aaa        864
Val  Gln  Lys  Tyr  Ile  Lys  Pro  Ser  Asp  Gly  Lys  Gln  Lys  Gly  Ala  Lys
     275                       280                       285
act  gag  tga                                                                    873
Thr  Glu
     290
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<210> 135
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<212> PRT
<213> Physcomitrella patens

<400> 135

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Met  Glu  Val  Val  Glu  Arg  Phe  Tyr  Gly  Glu  Leu  Asp  Gly  Lys  Val  Ser
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Gln  Gly  Val  Asn  Ala  Leu  Leu  Gly  Ser  Phe  Gly  Val  Glu  Leu  Thr  Asp
                 20                      25                      30
Thr  Pro  Thr  Thr  Lys  Gly  Leu  Pro  Leu  Val  Asp  Ser  Pro  Thr  Pro  Ile
            35                      40                      45
Val  Leu  Gly  Val  Ser  Val  Tyr  Leu  Thr  Ile  Val  Ile  Gly  Gly  Leu  Leu
     50                      55                      60
Trp  Ile  Lys  Ala  Arg  Asp  Leu  Lys  Pro  Arg  Ala  Ser  Glu  Pro  Phe  Leu
65                70                      75                      80
Leu  Gln  Ala  Leu  Val  Leu  Val  His  Asn  Leu  Phe  Cys  Phe  Ala  Leu  Ser
                 85                      90                      95
Leu  Tyr  Met  Cys  Val  Gly  Ile  Ala  Tyr  Gln  Ala  Ile  Thr  Trp  Arg  Tyr
            100                     105                     110
Ser  Leu  Trp  Gly  Asn  Ala  Tyr  Asn  Pro  Lys  His  Lys  Glu  Met  Ala  Ile
            115                     120                     125
Leu  Val  Tyr  Leu  Phe  Tyr  Met  Ser  Lys  Tyr  Val  Glu  Phe  Met  Asp  Thr
     130                     135                     140
Val  Ile  Met  Ile  Leu  Lys  Arg  Ser  Thr  Arg  Gln  Ile  Ser  Phe  Leu  His
145               150                     155                     160
Val  Tyr  His  His  Ser  Ser  Ile  Ser  Leu  Ile  Trp  Trp  Ala  Ile  Ala  His
                 165                     170                     175
His  Ala  Pro  Gly  Gly  Glu  Ala  Tyr  Trp  Ser  Ala  Ala  Leu  Asn  Ser  Gly
            180                     185                     190
Val  His  Val  Leu  Met  Tyr  Ala  Tyr  Tyr  Phe  Leu  Ala  Ala  Cys  Leu  Arg
            195                     200                     205
Ser  Ser  Pro  Lys  Leu  Lys  Asn  Lys  Tyr  Leu  Phe  Trp  Gly  Arg  Tyr  Leu
     210                     215                     220
Thr  Gln  Phe  Gln  Met  Phe  Gln  Phe  Met  Leu  Asn  Leu  Val  Gln  Ala  Tyr
225               230                     235                     240
Tyr  Asp  Met  Lys  Thr  Asn  Ala  Pro  Tyr  Pro  Gln  Trp  Leu  Ile  Lys  Ile
                 245                     250                     255
Leu  Phe  Tyr  Tyr  Met  Ile  Ser  Leu  Leu  Phe  Leu  Phe  Gly  Asn  Phe  Tyr
            260                     265                     270
Val  Gln  Lys  Tyr  Ile  Lys  Pro  Ser  Asp  Gly  Lys  Gln  Lys  Gly  Ala  Lys
            275                     280                     285
Thr  Glu
     290
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<210> 136
<211> 957
<212> DNA
<213> Mortierella alpina

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<400> 136

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Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
1               5                  10                  15
ttt atg gac ctt gcc acc gct atc ggt gtc cgg gcc gcg ccc tat gtc        96
Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
            20                  25                  30
gat cct ctc gag gcc gcg ctg gtg gcc cag gcc gag aag tac atc ccc       144
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
        35                  40                  45
acg att gtc cat cac acg cgt ggg ttc ctg gtc gcg gtg gag tcg cct       192
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
    50                  55                  60
ttg gcc cgt gag ctg ccg ttg atg aac ccg ttc cac gtg ctg ttg atc       240
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65                  70                  75                  80
gtg ctc gct tat ttg gtc acg gtc ttt gtg ggc atg cag atc atg aag       288
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
                85                  90                  95
aac ttt gag cgg ttc gag gtc aag acg ttt tcg ctc ctg cac aac ttt       336
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
            100                 105                 110
tgt ctg gtc tcg atc agc gcc tac atg tgc ggt ggg atc ctg tac gag       384
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
        115                 120                 125
gct tat cag gcc aac tat gga ctg ttt gag aac gct gct gat cat acc       432
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
    130                 135                 140
ttc aag ggt ctt cct atg gcc aag atg atc tgg ctc ttc tac ttc tcc       480
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145                 150                 155                 160
aag atc atg gag ttt gtc gac acc atg atc atg gtc ctc aag aag aac       528
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
                165                 170                 175
aac cgc cag atc tcc ttc ttg cac gtt tac cac cac agc tcc atc ttc       576
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
            180                 185                 190
acc atc tgg tgg ttg gtc acc ttt gtt gca ccc aac ggt gaa gcc tac       624
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
        195                 200                 205
ttc tct gct gcg ttg aac tcg ttc atc cat gtg atc atg tac ggc tac       672
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
    210                 215                 220
tac ttc ttg tcg gcc ttg ggc ttc aag cag gtg tcg ttc atc aag ttc       720
Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225                 230                 235                 240
tac atc acg cgc tcg cag atg aca cag ttc tgc atg atg tcg gtc cag       768
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
                245                 250                 255
tct tcc tgg gac atg tac gcc atg aag gtc ctt ggc cgc ccc gga tac       816
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
            260                 265                 270
ccc ttc ttc atc acg gct ctg ctt tgg ttc tac atg tgg acc atg ctc       864
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
        275                 280                 285
ggt ctc ttc tac aac ttt tac aga aag aac gcc aag ttg gcc aag cag       912
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
    290                 295                 300
gcc aag gcc gac gct gcc aag gag aag gca agg aag ttg cag taa           957
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
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<210> 137
<211> 318

<212> PRT
<213> Mortierella alpina

<400> 137
Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
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Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
20 25 30
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
35 40 45
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
50 55 60
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65 70 75 80
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
85 90 95
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
100 105 110
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
115 120 125
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
130 135 140
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145 150 155 160
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
165 170 175
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
180 185 190
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
195 200 205
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
210 215 220
Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225 230 235 240
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
245 250 255
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
260 265 270
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
275 280 285
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
290 295 300
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305 310 315

<210> 138
<211> 242
<212> PRT
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Tyr Leu xaa xaa Val xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
            20   25   30
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
        35   40   45
xaa His Asn xaa xaa xaa xaa xaa Leu Ser xaa Tyr Met xaa xaa Gly
    50   55   60
xaa xaa xaa xaa Ala xaa xaa xaa xaa Tyr xaa Leu xaa xaa Asn xaa
65   70   75   80
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
                85   90   95
xaa xaa Phe Tyr xaa Ser Lys xaa xaa Glu Phe xaa Asp Thr xaa Ile
            100   105   110
Met xaa Leu xaa xaa xaa xaa xaa Gln xaa xaa xaa Leu His xaa Tyr
        115   120   125
His His xaa xaa Ile xaa xaa Ile Trp Trp xaa xaa xaa xaa xaa Ala
    130   135   140
Pro Gly Gly xaa xaa Tyr xaa Ser Ala xaa xaa Asn Ser xaa xaa His
145   150   155   160
Val xaa Met Tyr xaa Tyr Tyr xaa Leu xaa xaa xaa xaa xaa xaa xaa
                165   170   175
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Thr xaa
            180   185   190
xaa Gln Met xaa Gln Phe xaa xaa xaa xaa xaa Gln xaa xaa Tyr xaa
        195   200   205
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Tyr Pro xaa xaa xaa
    210   215   220
xaa xaa xaa Leu xaa xaa Tyr Met xaa xaa xaa Leu xaa Leu Phe Gly
225   230   235   240
xaa Phe

<210> 139

<211> 60
<212> PRT
<213> Artificial sequence

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<223> xaa in position 2 is Phe or Trp
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<223> xaa in position 3 is Leu, Met or Val
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<223> xaa in position 6 is any amino acid
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<222> (7)..(7)
<223> xaa in position 7 is Phe or Ile
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<223> xaa in position 9 is any or no amino acid
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<223> xaa in position 11 is any or no amino acid
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<223> xaa in position 13 to 15 is any amino acid
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<222> (16)..(16)
<223> xaa in position 16 is any or no amino acid
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<222> (18)..(18)
<223> xaa in position 18 is Ile, Leu or Val
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<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Ser or Thr
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<222> (20)..(20)
<223> xaa in position 20 is Phe or Val
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<223> xaa in position 23 is Ile or Val
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<223> xaa in position 27 is Ala, Ser or Thr
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<222> (28)..(28)
<223> xaa in position 28 is Ser or Thr
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<223> xaa in position 30 to 31 is any amino acid

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<223> xaa in position 32 is Ile or Leu
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<223> xaa in position 36 is Ala, Ile or Val
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<223> xaa in position 42 is Gly or Asn
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<223> xaa in position 44 is Asp or Glu
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<223> xaa in position 47 is Phe, Trp or Tyr
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<223> xaa in position 58 is any amino acid
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<222> (59)..(59)
<223> xaa in position 59 is Leu or Met

<400> 139
Glu xaa xaa Asp Thr xaa xaa Met xaa Leu xaa Lys xaa xaa xaa xaa
 1   5   10   15

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Gln xaa xaa xaa Leu His xaa Tyr His His xaa xaa xaa xaa xaa xaa
                20                  25                  30
Trp xaa xaa xaa xaa xaa xaa Ala Pro xaa Gly xaa xaa Tyr xaa Ser
            35                  40                  45
xaa xaa xaa Asn Ser xaa xaa His Val xaa xaa Tyr
        50                  55                  60


<210> 140
<211> 22
<212> PRT
<213> Artificial sequence

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<223> xaa in position 5 is Cys or Ser
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<222> (6)..(6)
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<223> xaa in position 15 to 16 is any or no amino acid
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<223> xaa in position 18 is Gly, Ser or Thr
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<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Leu or Val
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Phe or Trp
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Glu, Gly or Asn

<400> 140
Ser xaa xaa Met xaa xaa Gly xaa xaa xaa xaa Ala xaa xaa xaa xaa
  1                 5                  10                  15
Tyr xaa xaa xaa xaa Asn
            20
```

<210> 141
<211> 18
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(3)
<223> xaa in position 2 to 3 is any amino acid
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any amino acid
<220>
<221> Variant
<222> (9)..(12)
<223> xaa in position 9 to 12 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Gly, Ile, Leu or Val
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ala or Ser
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is any amino acid
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Trp or Tyr
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Ala, Cys or Asp

<400> 141
Thr xaa xaa Gln Met xaa Gln Phe xaa xaa xaa xaa xaa Gln xaa xaa
  1   5   10   15
xaa xaa

<210> 142
<211> 25
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is any or no amino acid
<220>
<221> Variant
<222> (4)..(5)
<223> xaa in position 4 to 5 is any amino acid
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Phe, Ile or Leu
<220>
<221> Variant
<222> (7)..(8)
<223> xaa in position 7 to 8 is any amino acid
<220>

```
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Ile, Leu or Met
<220>
<221> Variant
<222> (11)..(12)
<223> xaa in position 11 to 12 is any amino acid
<220>
<221> Variant
<222> (14)..(15)
<223> xaa in position 14 to 15 is any amino acid
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Ser or Thr
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Leu or Met
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is any amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Phe or Leu
<220>
<221> Variant
<222> (22)..(23)
<223> xaa in position 22 to 23 is any amino acid
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is Phe or Tyr

<400> 142
Tyr xaa Pro xaa xaa xaa xaa xaa xaa Leu xaa xaa Tyr xaa xaa xaa
  1               5                  10                  15
xaa Leu xaa xaa Phe xaa xaa Phe xaa
            20                  25

<210> 143
<211> 879
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(879)

<400> 143
atg agt ggc tta cgt gca ccc aac ttt tta cac aga ttc tgg aca aag        48
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
tgg gac tac gcg att tcc aaa gtc gtc ttc acg tgt gcc gac agt ttt        96
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
cag tgg gac atc ggg cca gtg agt tcg agt acg gcg cat tta ccc gcc       144
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
att gaa tcc cct acc cca ctg gtg act agc ctc ttg ttc tac tta gtc       192
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
aca gtt ttc ttg tgg tat ggt cgt tta acc agg agt tca gac aag aaa       240
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
att aga gag cct acg tgg tta aga aga ttc ata ata tgt cat aat gcg       288
```

```
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
ttc ttg ata gtc ctc agt ctt tac atg tgc ctt ggt tgt gtg gcc caa      336
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
gcg tat cag aat gga tat act tta tgg ggt aat gaa ttc aag gcc acg      384
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
gaa act cag ctt gct ctc tac att tac att ttt tac gta agt aaa ata      432
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
tac gag ttt gta gat act tac att atg ctt ctc aag aat aac ttg cgg      480
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
caa gta agt ttc cta cac att tat cac cac agc acg att tcc ttt att      528
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
tgg tgg atc att gct cgg agg gct ccg ggt ggt gat gct tac ttc agc      576
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
gcg gcc ttg aac tca tgg gta cac gtg tgc atg tac acc tat tat cta      624
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
tta tca acc ctt att gga aaa gaa gat cct aag cgt tcc aac tac ctt      672
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
tgg tgg ggt cgc cac cta acg caa atg cag atg ctt cag ttt ttc ttc      720
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
aac gta ctt caa gcg ttg tac tgc gct tcg ttc tct acg tat ccc aag      768
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
ttt ttg tcc aaa att ctg ctc gtc tat atg atg agc ctt ctc ggc ttg      816
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
ttt ggg cat ttc tac tat tcc aag cac ata gca gca gct aag ctc cag      864
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
aaa aaa cag cag tga                                                  879
Lys Lys Gln Gln
    290
```

<210> 144
<211> 292
<212> PRT
<213> Ostreococcus tauri

<400> 144

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Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
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Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
Lys Lys Gln Gln
    290
```

<210> 145
<211> 831
<212> DNA
<213> Thraustochytrium sp

<220>
<221> CDS
<222> (1)..(831)

<400> 145

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atg gat gtc gtc gag cag caa tgg cgc cgc ttc gtg gac gcc gtg gac      48
Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                  10                  15
aac gga atc gtg gag ttc atg gag cat gag gag ccc aac aag ctg aac      96
Asn Gly Ile Val Glu Phe Met Glu His Glu Glu Pro Asn Lys Leu Asn
            20                  25                  30
gag ggc aag ctc tcc acc tcg acc gag gag atg atg gcg ctt atc gtc     144
Glu Gly Lys Leu Ser Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
ggc tac ctg gcg ttc gtg gtc ctc ggg tcc gcc ttc atg aag gcc ttt     192
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
gtc gat aag cct ttc gag ctc aag ttc ctc aag ctc gtg cac aac atc     240
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
ttc ctc acc ggt ctg tcc atg tac atg gcc acc gag tgc gcg cgc cag     288
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
gca tac ctc ggc ggc tac aag ctc ttt ggc aac ccg atg gag aag ggc     336
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
acc gag tcg cac gcc ccg ggc atg gcc aac atc atc tac atc ttc tac     384
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
gtg agc aag ttc ctc gaa ttc ctc gac acc gtc ttc atg atc ctc ggc     432
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
aag aag tgg aag cag ctc agc ttt ctc cac gtc tac cac cac gcg agc     480
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
atc agc ttc atc tgg ggc atc atc gcc cgc ttc gcg ccc ggt ggc gac     528
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
gcc tac ttc tct acc atc ctc aac agc agc gtg cat gtc gtg ctc tac     576
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
ggc tac tac gcc tcg acc acc ctc ggc tac acc ttc atg cgc ccg ctg     624
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
```

```
cgc ccg tac att acc acc att cag ctc acg cag ttc atg gcc atg gtc        672
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
gtc cag tcc gtc tat gac tac tac aac ccc tgc gac tac ccg cag ccc        720
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
ctc gtc aag ctg ctc ttc tgg tac atg ctc acc atg ctc ggc ctc ttc        768
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
ggc aac ttc ttc gtg cag cag tac ctc aag ccc aag gcg ccc aag aag        816
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
cag aag acc atc taa                                                831
Gln Lys Thr Ile
        275

<210> 146
<211> 276
<212> PRT
<213> Thraustochytrium sp

<400> 146
Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                   10                  15
Asn Gly Ile Val Glu Phe Met Glu His Glu Glu Pro Asn Lys Leu Asn
            20                  25                  30
Glu Gly Lys Leu Ser Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
Gln Lys Thr Ile
        275

<210> 147
<211> 1146
<212> DNA
<213> Leishmania major

<220>
<221> CDS
<222> (1)..(1146)
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<400> 147

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atg gtc tct ctg gag cag gcc gaa cag atc gcg gcc gcg atc gag gta        48
Met Val Ser Leu Glu Gln Ala Glu Gln Ile Ala Ala Ala Ile Glu Val
1               5                  10                  15
cct gac tgg gtc ttg aca aag tct gcg gcg ctg gtg tac agc tgc ttc        96
Pro Asp Trp Val Leu Thr Lys Ser Ala Ala Leu Val Tyr Ser Cys Phe
            20                  25                  30
ggc tcc gcc gcc aat gct ttt gaa agc agt atc aag atc aac ttc ccg       144
Gly Ser Ala Ala Asn Ala Phe Glu Ser Ser Ile Lys Ile Asn Phe Pro
        35                  40                  45
gcg cag cat gcg ttc gtg gag gcg tgg atg cgc gcg cgc tcc cac cct       192
Ala Gln His Ala Phe Val Glu Ala Trp Met Arg Ala Arg Ser His Pro
    50                  55                  60
ttt gcg gag cgc ctg ccg tac ctg aat ccg tgg cac gtt atc gcc tcg       240
Phe Ala Glu Arg Leu Pro Tyr Leu Asn Pro Trp His Val Ile Ala Ser
65                  70                  75                  80
ata ctg gcc tac ctc tcc ttg att gtc acc ttg cgc ctg ttg cat cgt       288
Ile Leu Ala Tyr Leu Ser Leu Ile Val Thr Leu Arg Leu Leu His Arg
                85                  90                  95
gta ctc ggt aag ttc tcg tgc cgc act ctc gga ttg gtg cac aac ctc       336
Val Leu Gly Lys Phe Ser Cys Arg Thr Leu Gly Leu Val His Asn Leu
            100                 105                 110
ggt ctc cat ctt ctc tcg ttg tac atg agc ctt ggt ctc atg atc agc       384
Gly Leu His Leu Leu Ser Leu Tyr Met Ser Leu Gly Leu Met Ile Ser
        115                 120                 125
gcg cgc gcc gcg ggg tac tcg ctc tgg aac aac gcg gtc ggc acc tcc       432
Ala Arg Ala Ala Gly Tyr Ser Leu Trp Asn Asn Ala Val Gly Thr Ser
    130                 135                 140
ccg gct gag tgg cgc att gcg aag ctg atc tgg ctc ttc tat gtc tcg       480
Pro Ala Glu Trp Arg Ile Ala Lys Leu Ile Trp Leu Phe Tyr Val Ser
145                 150                 155                 160
aag gtg gtg gaa tgg gtg gac acg gta att atg tta tta aag cag aac       528
Lys Val Val Glu Trp Val Asp Thr Val Ile Met Leu Leu Lys Gln Asn
                165                 170                 175
tac cac cag gtc acc ttc ctg cac gtg tat cac cac acg acg gtt ttt       576
Tyr His Gln Val Thr Phe Leu His Val Tyr His His Thr Thr Val Phe
            180                 185                 190
gtg ctg tgg tgg ctg gcg ttg ctg gtc gct cct ggc ggc gag tcg tac       624
Val Leu Trp Trp Leu Ala Leu Leu Val Ala Pro Gly Gly Glu Ser Tyr
        195                 200                 205
tac agc gcc atg gtg aac tct ggc gtc cac gtt ttc atg tac ggg tac       672
Tyr Ser Ala Met Val Asn Ser Gly Val His Val Phe Met Tyr Gly Tyr
    210                 215                 220
tac ttt ctc acg ctg ctc ttc cca tcc ggc atc gtg cgc gac gtc ttg       720
Tyr Phe Leu Thr Leu Leu Phe Pro Ser Gly Ile Val Arg Asp Val Leu
225                 230                 235                 240
agc aag ttc aag ttt gcc att acg aag ggc cag atg tgg cag ttc gtc       768
Ser Lys Phe Lys Phe Ala Ile Thr Lys Gly Gln Met Trp Gln Phe Val
                245                 250                 255
ttc aac tgc cta cag tcc gcg tac gac ctc gtg tgg gtg ccg cgg gaa       816
Phe Asn Cys Leu Gln Ser Ala Tyr Asp Leu Val Trp Val Pro Arg Glu
            260                 265                 270
gag ctc aag tac agc gcg gag ctg ctg cag atc ctc ttc tgg tac atg       864
Glu Leu Lys Tyr Ser Ala Glu Leu Leu Gln Ile Leu Phe Trp Tyr Met
        275                 280                 285
atc tcc ctc ttg gcg ctc ttt ggc aac ttc ttg gtg aag aac aag aag       912
Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Leu Val Lys Asn Lys Lys
    290                 295                 300
ttc tcg cac cgc cgc tgc gtt gat gcc gcg act gct tcg ggc gcg aag       960
Phe Ser His Arg Arg Cys Val Asp Ala Ala Thr Ala Ser Gly Ala Lys
305                 310                 315                 320
gag gac acg gcg gcg agg tcc cac ggc gac cgc acc cac aga acc cgt      1008
Glu Asp Thr Ala Ala Arg Ser His Gly Asp Arg Thr His Arg Thr Arg
                325                 330                 335
gtg aag gct ggc atg acc aac atg caa ctg gag agg ctg aag aat gag      1056
Val Lys Ala Gly Met Thr Asn Met Gln Leu Glu Arg Leu Lys Asn Glu
            340                 345                 350
aag tcc acg gag atg aag ctg ctg atg cgc aag aac ggc aac ggc aac      1104
```

```
Lys Ser Thr Glu Met Lys Leu Leu Met Arg Lys Asn Gly Asn Gly Asn
        355                 360                 365
gga caa aaa gcg tcg ctc cag gcc atg gca ggc agt cga tga                1146
Gly Gln Lys Ala Ser Leu Gln Ala Met Ala Gly Ser Arg
    370                 375                 380

<210> 148
<211> 381
<212> PRT
<213> Leishmania major

<400> 148
Met Val Ser Leu Glu Gln Ala Glu Gln Ile Ala Ala Ala Ile Glu Val
1               5                  10                  15
Pro Asp Trp Val Leu Thr Lys Ser Ala Ala Leu Val Tyr Ser Cys Phe
            20                  25                  30
Gly Ser Ala Ala Asn Ala Phe Glu Ser Ser Ile Lys Ile Asn Phe Pro
        35                  40                  45
Ala Gln His Ala Phe Val Glu Ala Trp Met Arg Ala Arg Ser His Pro
    50                  55                  60
Phe Ala Glu Arg Leu Pro Tyr Leu Asn Pro Trp His Val Ile Ala Ser
65                  70                  75                  80
Ile Leu Ala Tyr Leu Ser Leu Ile Val Thr Leu Arg Leu Leu His Arg
                85                  90                  95
Val Leu Gly Lys Phe Ser Cys Arg Thr Leu Gly Leu Val His Asn Leu
            100                 105                 110
Gly Leu His Leu Leu Ser Leu Tyr Met Ser Leu Gly Leu Met Ile Ser
        115                 120                 125
Ala Arg Ala Ala Gly Tyr Ser Leu Trp Asn Asn Ala Val Gly Thr Ser
    130                 135                 140
Pro Ala Glu Trp Arg Ile Ala Lys Leu Ile Trp Leu Phe Tyr Val Ser
145                 150                 155                 160
Lys Val Val Glu Trp Val Asp Thr Val Ile Met Leu Leu Lys Gln Asn
                165                 170                 175
Tyr His Gln Val Thr Phe Leu His Val Tyr His His Thr Thr Val Phe
            180                 185                 190
Val Leu Trp Trp Leu Ala Leu Leu Val Ala Pro Gly Gly Glu Ser Tyr
        195                 200                 205
Tyr Ser Ala Met Val Asn Ser Gly Val His Val Phe Met Tyr Gly Tyr
    210                 215                 220
Tyr Phe Leu Thr Leu Leu Phe Pro Ser Gly Ile Val Arg Asp Val Leu
225                 230                 235                 240
Ser Lys Phe Lys Phe Ala Ile Thr Lys Gly Gln Met Trp Gln Phe Val
                245                 250                 255
Phe Asn Cys Leu Gln Ser Ala Tyr Asp Leu Val Trp Val Pro Arg Glu
            260                 265                 270
Glu Leu Lys Tyr Ser Ala Glu Leu Leu Gln Ile Leu Phe Trp Tyr Met
        275                 280                 285
Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Leu Val Lys Asn Lys Lys
    290                 295                 300
Phe Ser His Arg Arg Cys Val Asp Ala Ala Thr Ala Ser Gly Ala Lys
305                 310                 315                 320
Glu Asp Thr Ala Ala Arg Ser His Gly Asp Arg Thr His Arg Thr Arg
                325                 330                 335
Val Lys Ala Gly Met Thr Asn Met Gln Leu Glu Arg Leu Lys Asn Glu
            340                 345                 350
Lys Ser Thr Glu Met Lys Leu Leu Met Arg Lys Asn Gly Asn Gly Asn
        355                 360                 365
Gly Gln Lys Ala Ser Leu Gln Ala Met Ala Gly Ser Arg
    370                 375                 380

<210> 149
<211> 879
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
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<222> (1)..(879)

<400> 149

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atg agt ggc tta cgt gca ccc aac ttt tta cac aga ttc tgg aca aag        48
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
tgg gac tac gcg att tcc aaa gtc gtc ttc acg tgt gcc gac agt ttt        96
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
cag tgg gac atc ggg cca gtg agt tcg agt acg gcg cat tta ccc gcc       144
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
att gaa tcc cct acc cca ctg gtg act agc ctc ttg ttc tac tta gtc       192
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
aca gtt ttc ttg tgg tat ggt cgt tta acc agg agt tca gac aag aaa       240
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
att aga gag cct acg tgg tta aga aga ttc ata ata tgt cat aat gcg       288
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
ttc ttg ata gtc ctc agt ctt tac atg tgc ctt ggt tgt gtg gcc caa       336
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
gcg tat cag aat gga tat act tta tgg ggt aat gaa ttc aag gcc acg       384
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
gaa act cag ctt gct ctc tac att tac att ttt tac gta agt aaa ata       432
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
tac gag ttt gta gat act tac att atg ctt ctc aag aat aac ttg cgg       480
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
caa gta agt ttc cta cac att tat cac cac agc acg att tcc ttt att       528
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
tgg tgg atc att gct cgg agg gct ccg ggt ggt gat gct tac ttc agc       576
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
gcg gcc ttg aac tca tgg gta cac gtg tgc atg tac acc tat tat cta       624
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
tta tca acc ctt att gga aaa gaa gat cct aag cgt tcc aac tac ctt       672
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
tgg tgg ggt cgc cac cta acg caa atg cag atg ctt cag ttt ttc ttc       720
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
aac gta ctt caa gcg ttg tac tgc gct tcg ttc tct acg tat ccc aag       768
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
ttt ttg tcc aaa att ctg ctc gtc tat atg atg agc ctt ctc ggc ttg       816
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
ttt ggg cat ttc tac tat tcc aag cac ata gca gca gct aag ctc cag       864
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
aaa aaa cag cag tga                                                   879
Lys Lys Gln Gln
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<210> 150
<211> 292
<212> PRT
<213> Ostreococcus tauri

<400> 150

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Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
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Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
Lys Lys Gln Gln
    290
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<210> 151
<211> 873
<212> DNA
<213> Physcomitrella patens

<220>
<221> CDS
<222> (1)..(873)

<400> 151

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Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
1               5                  10                  15
cag ggc gtg aat gca ttg ctg ggt agt ttt ggg gtg gag ttg acg gat        96
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30
acg ccc act acc aaa ggc ttg ccc ctc gtt gac agt ccc aca ccc atc       144
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45
gtc ctc ggt gtt tct gta tac ttg act att gtc att gga ggg ctt ttg       192
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60
tgg ata aag gcc agg gat ctg aaa ccg cgc gcc tcg gag cca ttt ttg       240
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80
ctc caa gct ttg gtg ctt gtg cac aac ctg ttc tgt ttt gcg ctc agt       288
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95
ctg tat atg tgc gtg ggc atc gct tat cag gct att acc tgg cgg tac       336
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
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                100                 105                 110
tct ctc tgg ggc aat gca tac aat cct aaa cat aaa gag atg gcg att        384
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125
ctg gta tac ttg ttc tac atg tct aag tac gtg gaa ttc atg gat acc        432
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140
gtt atc atg ata ctg aag cgc agc acc agg caa ata agc ttc ctc cac        480
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160
gtt tat cat cat tct tca att tcc ctc att tgg tgg gct att gct cat        528
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175
cac gct cct ggc ggt gaa gca tat tgg tct gcg gct ctg aac tca gga        576
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190
gtg cat gtt ctc atg tat gcg tat tac ttc ttg gct gcc tgc ctt cga        624
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205
agt agc cca aag tta aaa aat aag tac ctt ttt tgg ggc agg tac ttg        672
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220
aca caa ttc caa atg ttc cag ttt atg ctg aac tta gtg cag gct tac        720
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240
tac gac atg aaa acg aat gcg cca tat cca caa tgg ctg atc aag att        768
Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255
ttg ttc tac tac atg atc tcg ttg ctg ttt ctt ttc ggc aat ttt tac        816
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
            260                 265                 270
gta caa aaa tac atc aaa ccc tct gac gga aag caa aag gga gct aaa        864
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
        275                 280                 285
act gag tga                                                           873
Thr Glu
    290
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<210> 152
<211> 290
<212> PRT
<213> Physcomitrella patens

<400> 152

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Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
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Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190
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Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240
Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
            260                 265                 270
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
        275                 280                 285
Thr Glu
    290

<210> 153
<211> 957
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(957)

<400> 153
atg gag tcg att gcg cca ttc ctc cca tca aag atg ccg caa gat ctg        48
Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
1               5                   10                  15
ttt atg gac ctt gcc acc gct atc ggt gtc cgg gcc gcg ccc tat gtc        96
Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
            20                  25                  30
gat cct ctc gag gcc gcg ctg gtg gcc cag gcc gag aag tac atc ccc       144
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
        35                  40                  45
acg att gtc cat cac acg cgt ggg ttc ctg gtc gcg gtg gag tcg cct       192
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
    50                  55                  60
ttg gcc cgt gag ctg ccg ttg atg aac ccg ttc cac gtg ctg ttg atc       240
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65                  70                  75                  80
gtg ctc gct tat ttg gtc acg gtc ttt gtg ggc atg cag atc atg aag       288
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
                85                  90                  95
aac ttt gag cgg ttc gag gtc aag acg ttt tcg ctc ctg cac aac ttt       336
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
            100                 105                 110
tgt ctg gtc tcg atc agc gcc tac atg tgc ggt ggg atc ctg tac gag       384
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
        115                 120                 125
gct tat cag gcc aac tat gga ctg ttt gag aac gct gct gat cat acc       432
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
    130                 135                 140
ttc aag ggt ctt cct atg gcc aag atg atc tgg ctc ttc tac ttc tcc       480
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145                 150                 155                 160
aag atc atg gag ttt gtc gac acc atg atc atg gtc ctc aag aag aac       528
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
                165                 170                 175
aac cgc cag atc tcc ttc ttg cac gtt tac cac cac agc tcc atc ttc       576
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
            180                 185                 190
acc atc tgg tgg ttg gtc acc ttt gtt gca ccc aac ggt gaa gcc tac       624
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
        195                 200                 205
ttc tct gct gcg ttg aac tcg ttc atc cat gtg atc atg tac ggc tac       672
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
    210                 215                 220
tac ttc ttg tcg gcc ttg ggc ttc aag cag gtg tcg ttc atc aag ttc       720
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Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225                 230                 235                 240
tac atc acg cgc tcg cag atg aca cag ttc tgc atg atg tcg gtc cag        768
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
                245                 250                 255
tct tcc tgg gac atg tac gcc atg aag gtc ctt ggc cgc ccc gga tac        816
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
            260                 265                 270
ccc ttc ttc atc acg gct ctg ctt tgg ttc tac atg tgg acc atg ctc        864
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
        275                 280                 285
ggt ctc ttc tac aac ttt tac aga aag aac gcc aag ttg gcc aag cag        912
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
    290                 295                 300
gcc aag gcc gac gct gcc aag gag aag gca agg aag ttg cag taa            957
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305                 310                 315

<210> 154
<211> 318
<212> PRT
<213> Mortierella alpina

<400> 154
Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
1               5                  10                  15
Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
            20                  25                  30
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
        35                  40                  45
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
    50                  55                  60
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65                  70                  75                  80
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
                85                  90                  95
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
            100                 105                 110
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
        115                 120                 125
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
    130                 135                 140
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145                 150                 155                 160
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
                165                 170                 175
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
            180                 185                 190
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
        195                 200                 205
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
    210                 215                 220
Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225                 230                 235                 240
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
                245                 250                 255
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
            260                 265                 270
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
        275                 280                 285
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
    290                 295                 300
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305                 310                 315


<210> 155
<211> 236
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<212> PRT
<213> Artificial sequence

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<222> (2)..(8)
<223> xaa in position 2 to 8 is any amino acid
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<222> (10)..(10)
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<222> (13)..(14)
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<223> xaa in position 16 to 36 is any amino acid
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<222> (37)..(43)
<223> xaa in position 37 to 43 is any or no amino acid
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<223> xaa in position 49 to 50 is any amino acid
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<222> (53)..(53)
<223> xaa in position 53 is any amino acid
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<223> xaa in position 56 to 57 is any amino acid
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<223> xaa in position 59 to 62 is any amino acid
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<223> xaa in position 64 to 67 is any amino acid
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<222> (69)..(69)
<223> xaa in position 69 is any amino acid
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<223> xaa in position 71 to 72 is any amino acid
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<222> (74)..(82)
<223> xaa in position 74 to 82 is any amino acid
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<223> xaa in position 83 to 86 is any or no amino acid
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<223> xaa in position 88 to 89 is any amino acid
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<223> xaa in position 91 to 92 is any amino acid
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<222> (95)..(95)
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<222> (98)..(99)
<223> xaa in position 98 to 99 is any amino acid
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<223> xaa in position 102 is any amino acid
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<223> xaa in position 108 is any amino acid
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<223> xaa in position 111 to 114 is any amino acid
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<223> xaa in position 116 is any amino acid
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<223> xaa in position 121 is any amino acid
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<223> xaa in position 125 to 126 is any amino acid
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<223> xaa in position 128 to 129 is any amino acid
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<223> xaa in position 133 to 137 is any amino acid
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<222> (142)..(142)
<223> xaa in position 142 is any amino acid
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<222> (145)..(145)
<223> xaa in position 145 is any amino acid
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<223> xaa in position 148 is any amino acid
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<223> xaa in position 152 is any amino acid
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<222> (156)..(156)
<223> xaa in position 156 is any amino acid
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<223> xaa in position 164 to 165 is any amino acid
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<223> xaa in position 167 to 178 is any amino acid
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<223> xaa in position 179 to 184 is any or no amino acid
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<223> xaa in position 193 to 197 is any amino acid
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<223> xaa in position 235 is any amino acid

<400> 155
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20 25 30
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa His Asn xaa xaa Leu
35 40 45
xaa xaa Leu Ser xaa Tyr Met xaa xaa Gly xaa xaa xaa xaa Ala xaa
50 55 60
xaa xaa xaa Tyr xaa Leu xaa xaa Asn xaa xaa xaa xaa xaa xaa xaa
65 70 75 80
xaa xaa xaa xaa xaa xaa Ala xaa xaa Ile xaa xaa Phe Tyr xaa Ser
85 90 95
Lys xaa xaa Glu Phe xaa Asp Thr xaa Ile Met xaa Leu Lys xaa xaa
100 105 110
xaa xaa Gln xaa Ser Phe Leu His xaa Tyr His His xaa xaa Ile xaa
115 120 125
xaa Ile Trp Trp xaa xaa xaa xaa xaa Ala Pro Gly Gly xaa Ala Tyr
130 135 140
xaa Ser Ala xaa Leu Asn Ser xaa Val His Val xaa Met Tyr xaa Tyr
145 150 155 160
Tyr xaa Leu xaa xaa Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
165 170 175
xaa xaa xaa xaa xaa xaa xaa xaa Thr xaa xaa Gln Met xaa Gln Phe
180 185 190
xaa xaa xaa xaa xaa Gln xaa xaa Tyr xaa xaa xaa xaa xaa xaa xaa
195 200 205
xaa xaa xaa xaa xaa Tyr Pro xaa xaa Leu xaa xaa xaa Leu xaa xaa
210 215 220
Tyr Met xaa xaa xaa Leu xaa Leu Phe Gly xaa Phe
225 230 235

<210> 156
<211> 59
<212> PRT
<213> Artificial sequence

<220>
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<223> xaa in position 5 is any amino acid
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<222> (6)..(6)
<223> xaa in position 6 is Phe or Ile
<220>
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<222> (8)..(8)
<223> xaa in position 8 is Ile, Leu or Val
<220>
<221> Variant
<222> (10)..(13)
<223> xaa in position 10 to 13 is any amino acid
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<222> (14)..(14)
<223> xaa in position 14 is Lys or Arg
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<222> (16)..(16)
<223> xaa in position 16 is Ile, Leu or Val
<220>
<221> Variant

<222> (21)..(21)
<223> xaa in position 21 is Ile or Val
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<222> (25)..(25)
<223> xaa in position 25 is Ala or Ser
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<222> (26)..(26)
<223> xaa in position 26 is Ser or Thr
<220>
<221> Variant
<222> (28)..(29)
<223> xaa in position 28 to 29 is any amino acid
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<222> (32)..(32)
<223> xaa in position 32 is any amino acid
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<222> (33)..(33)
<223> xaa in position 33 is Ala, Ile or Leu
<220>
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<222> (34)..(34)
<223> xaa in position 34 is Ile or Val
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<221> Variant
<222> (35)..(35)
<223> xaa in position 35 is Ala or Thr
<220>
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<222> (36)..(37)
<223> xaa in position 36 to 37 is any amino acid
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<222> (40)..(40)
<223> xaa in position 40 is Gly or Asn
<220>
<221> Variant
<222> (42)..(42)
<223> xaa in position 42 is Asp or Glu
<220>
<221> Variant
<222> (45)..(45)
<223> xaa in position 45 is Phe or Trp
<220>
<221> Variant
<222> (47)..(47)
<223> xaa in position 47 is Ala or Thr
<220>
<221> Variant
<222> (48)..(48)
<223> xaa in position 48 is Ala or Ile
<220>
<221> Variant
<222> (52)..(52)
<223> xaa in position 52 is any amino acid
<220>
<221> Variant
<222> (53)..(53)
<223> xaa in position 53 is Ile or Val
<220>
<221> Variant
<222> (56)..(56)
<223> xaa in position 56 is any amino acid
<220>
<221> Variant

<222> (57)..(57)
<223> xaa in position 57 is Leu or Met
<220>
<221> Variant
<222> (59)..(59)
<223> xaa in position 59 is Ala, Gly or Thr

<400> 156
Phe xaa Asp Thr xaa xaa Met xaa Leu xaa xaa xaa xaa xaa Gln xaa
1 5 10 15
Ser Phe Leu His xaa Tyr His His xaa xaa Ile xaa xaa Ile Trp xaa
20 25 30
xaa xaa xaa xaa xaa Ala Pro xaa Gly xaa Ala Tyr xaa Ser xaa xaa
35 40 45
Leu Asn Ser xaa xaa His Val xaa xaa Tyr xaa
50 55

<210> 157
<211> 22
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Leu or Met
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ala, Cys or Ser
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any amino acid
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Glu or Gly
<220>
<221> Variant
<222> (8)..(10)
<223> xaa in position 8 to 10 is any amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is Gln or Ser
<220>
<221> Variant
<222> (13)..(16)
<223> xaa in position 13 to 16 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Phe or Trp
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Gly or Asn

<400> 157
Ser xaa Tyr Met xaa xaa xaa xaa xaa xaa xaa Ala xaa xaa xaa xaa
1 5 10 15
Tyr xaa Leu xaa xaa Asn

20

<210> 158
<211> 16
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Leu or Met
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is any amino acid
<220>
<221> Variant
<222> (6)..(8)
<223> xaa in position 6 to 8 is any amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is any or no amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is any or no amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Ala or Ser
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Trp or Tyr
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Cys or Asp

<400> 158
Gln xaa xaa Gln Phe xaa xaa xaa xaa Val xaa Gln xaa xaa xaa xaa
1 5 10 15

<210> 159
<211> 24
<212> PRT
<213> Artificial sequence

<220>
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<222> (3)..(4)
<223> xaa in position 3 to 4 is any amino acid
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<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ile or Leu
<220>
<221> Variant
<222> (6)..(7)
<223> xaa in position 6 to 7 is any amino acid

<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Ile or Leu
<220>
<221> Variant
<222> (10)..(11)
<223> xaa in position 10 to 11 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ser or Thr
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Leu or Met
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (21)..(22)
<223> xaa in position 21 to 22 is any amino acid
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Phe or Tyr

<400> 159
Tyr Pro xaa xaa xaa xaa xaa xaa Leu xaa xaa Tyr Met xaa xaa xaa
 1   5   10   15
Leu xaa Leu Phe xaa xaa Phe xaa
            20

<210> 160
<211> 8
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Trp or Tyr
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is Ile or Leu
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Phe or Val

<400> 160
Ile xaa xaa Phe Tyr xaa Ser Lys
 1   5

<210> 161
<211> 755
<212> DNA
<213> Cauliflower mosaic virus

<400> 161

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cctgcaggtc aacatggtgg agcacgacac acttgtctac tccaaaaata tcaaagatac      60
agtctcagaa gaccaaaggg caattgagac ttttcaacaa agggtaatat ccggaaacct      120
cctcggattc cattgcccag ctatctgtca ctttattgtg aagatagtgg aaaaggaagg      180
tggctcctac aaatgccatc attgcgataa aggaaaggcc atcgttgaag atgcctctgc      240
cgacagtggt cccaaagatg gacccccacc cacgaggagc atcgtggaaa aagaagacgt      300
tccaaccacg tcttcaaagc aagtggattg atgtgataac atggtggagc acgacacact      360
tgtctactcc aaaaatatca aagatacagt ctcagaagac caaagggcaa ttgagacttt      420
tcaacaaagg gtaatatccg gaaacctcct cggattccat tgcccagcta tctgtcactt      480
tattgtgaag atagtggaaa aggaaggtgg ctcctacaaa tgccatcatt gcgataaagg      540
aaaggccatc gttgaagatg cctctgccga cagtggtccc aaagatggac ccccacccac      600
gaggagcatc gtggaaaaag aagacgttcc aaccacgtct tcaaagcaag tggattgatg      660
tgatatctcc actgacgtaa gggatgacgc acaatcccac tatccttcgc aagacccttc      720
ctctatataa ggaagttcat ttcatttgga gagga                                 755

<210> 162
<211> 211
<212> DNA
<213> Cauliflower mosaic virus

<400> 162
agtccgcaaa aatcaccagt ctctctctac aaatctatct ctctctattt ttctccagaa      60
taatgtgtga gtagttccca gataagggaa ttagggttct tatagggttt cgctcatgtg      120
ttgagcatat aagaaaccct tagtatgtat ttgtatttgt aaaatacttc tatcaataaa      180
atttctaatt cctaaaacca aaatccagtg a                                     211

<210> 163
<211> 819
<212> DNA
<213> Thalassiosira pseudonana

<220>
<221> CDS
<222> (1)..(819)

<400> 163
atg gac gcc tac aac gct gca atg gat aag atc ggt gcc gcc atc atc        48
Met Asp Ala Tyr Asn Ala Ala Met Asp Lys Ile Gly Ala Ala Ile Ile
1               5                  10                  15
gat tgg tct gat ccc gat gga aag ttc cgt gcc gat aga gag gac tgg        96
Asp Trp Ser Asp Pro Asp Gly Lys Phe Arg Ala Asp Arg Glu Asp Trp
            20                  25                  30
tgg ctc tgc gac ttc cgt agc gcc atc acc atc gcc ctc atc tac atc       144
Trp Leu Cys Asp Phe Arg Ser Ala Ile Thr Ile Ala Leu Ile Tyr Ile
        35                  40                  45
gcc ttc gtc atc ctc ggt tcc gcc gtc atg caa tcc ctc ccc gca atg       192
Ala Phe Val Ile Leu Gly Ser Ala Val Met Gln Ser Leu Pro Ala Met
    50                  55                  60
gat ccc tac ccc atc aaa ttc ctc tac aac gtc tcc caa atc ttc ctt       240
Asp Pro Tyr Pro Ile Lys Phe Leu Tyr Asn Val Ser Gln Ile Phe Leu
65                  70                  75                  80
tgt gcc tac atg act gtc gag gcg gga ttt ttg gcc tac cgc aat gga       288
Cys Ala Tyr Met Thr Val Glu Ala Gly Phe Leu Ala Tyr Arg Asn Gly
                85                  90                  95
tat acc gtc atg cct tgc aat cat ttc aat gtg aat gat cct ccc gtg       336
Tyr Thr Val Met Pro Cys Asn His Phe Asn Val Asn Asp Pro Pro Val
            100                 105                 110
gcg aat ctt ctt tgg ttg ttt tat att tcc aag gtg tgg gac ttt tgg       384
Ala Asn Leu Leu Trp Leu Phe Tyr Ile Ser Lys Val Trp Asp Phe Trp
        115                 120                 125
gat acc att ttc att gtg ttg ggg aag aag tgg cgt caa tta tct ttc       432
Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gln Leu Ser Phe
    130                 135                 140
ttg cat gta tac cat cac acc atc ttt cta ttc tat tgg ctg aat           480
Leu His Val Tyr His His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
145                 150                 155                 160
gcc aat gtc ttg tac gat ggt gac atc ttc ctt acc atc ttg ctc aat       528
Ala Asn Val Leu Tyr Asp Gly Asp Ile Phe Leu Thr Ile Leu Leu Asn
                165                 170                 175
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gga ttc atc cac acg gtg atg tac acg tat tac ttc atc tgt atg cat        576
Gly Phe Ile His Thr Val Met Tyr Thr Tyr Tyr Phe Ile Cys Met His
                180                 185                 190
acc aaa gat tcc aag acg ggc aag agt ctt cct ata tgg tgg aag tcg        624
Thr Lys Asp Ser Lys Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser
        195                 200                 205
agt ttg acg gcg ttt cag ttg ttg caa ttc act atc atg atg agt cag        672
Ser Leu Thr Ala Phe Gln Leu Leu Gln Phe Thr Ile Met Met Ser Gln
    210                 215                 220
gct acc tac ctt gtc ttc cac ggg tgt gat aag gtg tcg ctt cgt atc        720
Ala Thr Tyr Leu Val Phe His Gly Cys Asp Lys Val Ser Leu Arg Ile
225                 230                 235                 240
acg att gtg tac ttt gtg tcc ctt ttg agt ttg ttc ttc ctt ttt gct        768
Thr Ile Val Tyr Phe Val Ser Leu Leu Ser Leu Phe Phe Leu Phe Ala
                245                 250                 255
cag ttc ttt gtg caa tca tac atg gca ccc aaa aag aag aag agt gct        816
Gln Phe Phe Val Gln Ser Tyr Met Ala Pro Lys Lys Lys Lys Ser Ala
            260                 265                 270
tag                                                                    819
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<210> 164
<211> 272
<212> PRT
<213> Thalassiosira pseudonana

<400> 164

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Met Asp Ala Tyr Asn Ala Ala Met Asp Lys Ile Gly Ala Ala Ile Ile
1               5                  10                  15
Asp Trp Ser Asp Pro Asp Gly Lys Phe Arg Ala Asp Arg Glu Asp Trp
            20                  25                  30
Trp Leu Cys Asp Phe Arg Ser Ala Ile Thr Ile Ala Leu Ile Tyr Ile
        35                  40                  45
Ala Phe Val Ile Leu Gly Ser Ala Val Met Gln Ser Leu Pro Ala Met
    50                  55                  60
Asp Pro Tyr Pro Ile Lys Phe Leu Tyr Asn Val Ser Gln Ile Phe Leu
65                  70                  75                  80
Cys Ala Tyr Met Thr Val Glu Ala Gly Phe Leu Ala Tyr Arg Asn Gly
                85                  90                  95
Tyr Thr Val Met Pro Cys Asn His Phe Asn Val Asn Asp Pro Pro Val
            100                 105                 110
Ala Asn Leu Leu Trp Leu Phe Tyr Ile Ser Lys Val Trp Asp Phe Trp
        115                 120                 125
Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gln Leu Ser Phe
    130                 135                 140
Leu His Val Tyr His His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
145                 150                 155                 160
Ala Asn Val Leu Tyr Asp Gly Asp Ile Phe Leu Thr Ile Leu Leu Asn
                165                 170                 175
Gly Phe Ile His Thr Val Met Tyr Thr Tyr Tyr Phe Ile Cys Met His
            180                 185                 190
Thr Lys Asp Ser Lys Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser
        195                 200                 205
Ser Leu Thr Ala Phe Gln Leu Leu Gln Phe Thr Ile Met Met Ser Gln
    210                 215                 220
Ala Thr Tyr Leu Val Phe His Gly Cys Asp Lys Val Ser Leu Arg Ile
225                 230                 235                 240
Thr Ile Val Tyr Phe Val Ser Leu Leu Ser Leu Phe Phe Leu Phe Ala
                245                 250                 255
Gln Phe Phe Val Gln Ser Tyr Met Ala Pro Lys Lys Lys Lys Ser Ala
            260                 265                 270
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<210> 165
<211> 837
<212> DNA
<213> Phaeodactylum tricornutum

<220>
<221> CDS
<222> (1)..(837)

<400> 165

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atg atg gta cct tca agt tat gac gag tat atc gtc atg gtc aac gac        48
Met Met Val Pro Ser Ser Tyr Asp Glu Tyr Ile Val Met Val Asn Asp
1               5                  10                  15
ctt ggc gac tct att ctg agc tgg gcc gac cct gat cac tat cgt gga        96
Leu Gly Asp Ser Ile Leu Ser Trp Ala Asp Pro Asp His Tyr Arg Gly
            20                  25                  30
cat acc gag gga tgg gag ttc act gac ttt tct gct gct ttt agc att       144
His Thr Glu Gly Trp Glu Phe Thr Asp Phe Ser Ala Ala Phe Ser Ile
        35                  40                  45
gcc gtc gcg tac ctc ctg ttt gtc ttt gtt gga tct ctc att atg agt       192
Ala Val Ala Tyr Leu Leu Phe Val Phe Val Gly Ser Leu Ile Met Ser
    50                  55                  60
atg gga gtc ccc gca att gac cct tat ccg ctc aag ttt gtc tac aat       240
Met Gly Val Pro Ala Ile Asp Pro Tyr Pro Leu Lys Phe Val Tyr Asn
65                  70                  75                  80
gtt tca cag att atg ctt tgt gct tac atg acc att gaa gcc agt ctt       288
Val Ser Gln Ile Met Leu Cys Ala Tyr Met Thr Ile Glu Ala Ser Leu
                85                  90                  95
cta gct tat cgt aac ggc tac aca ttc tgg cct tgc aac gat tgg gac       336
Leu Ala Tyr Arg Asn Gly Tyr Thr Phe Trp Pro Cys Asn Asp Trp Asp
            100                 105                 110
ttt gaa aag ccg cct atc gct aag ctc ctc tgg ctc ttt tac gtt tcc       384
Phe Glu Lys Pro Pro Ile Ala Lys Leu Leu Trp Leu Phe Tyr Val Ser
        115                 120                 125
aaa att tgg gat ttt tgg gac acc atc ttt att gtt ctc ggg aag aag       432
Lys Ile Trp Asp Phe Trp Asp Thr Ile Phe Ile Val Leu Gly Lys Lys
    130                 135                 140
tgg cgt caa ctt tcc ttc ctg cac gtc tac cat cac acc acc atc ttt       480
Trp Arg Gln Leu Ser Phe Leu His Val Tyr His His Thr Thr Ile Phe
145                 150                 155                 160
ctc ttc tac tgg ttg aat gca cat gta aac ttt gat ggt gat att ttc       528
Leu Phe Tyr Trp Leu Asn Ala His Val Asn Phe Asp Gly Asp Ile Phe
                165                 170                 175
ctc acc atc gtc ttg aac ggt ttc atc cac acc gtc atg tac acg tac       576
Leu Thr Ile Val Leu Asn Gly Phe Ile His Thr Val Met Tyr Thr Tyr
            180                 185                 190
tac ttc att tgc atg cac acc aag gtc cca gag acc ggc aaa tcc ttg       624
Tyr Phe Ile Cys Met His Thr Lys Val Pro Glu Thr Gly Lys Ser Leu
        195                 200                 205
ccc att tgg tgg aaa tct agt ttg aca agc atg cag ctg gtg cag ttc       672
Pro Ile Trp Trp Lys Ser Ser Leu Thr Ser Met Gln Leu Val Gln Phe
    210                 215                 220
atc acg atg atg acg cag gct atc atg atc ttg tac aag ggc tgt gct       720
Ile Thr Met Met Thr Gln Ala Ile Met Ile Leu Tyr Lys Gly Cys Ala
225                 230                 235                 240
gct ccc cat agc cgg gtg gtg aca tcg tac ttg gtt tac att ttg tcg       768
Ala Pro His Ser Arg Val Val Thr Ser Tyr Leu Val Tyr Ile Leu Ser
                245                 250                 255
ctc ttt att ttg ttc gcc cag ttc ttt gtc agc tca tac ctc aag ccg       816
Leu Phe Ile Leu Phe Ala Gln Phe Phe Val Ser Ser Tyr Leu Lys Pro
            260                 265                 270
aag aag aag aag aca gct taa                                           837
Lys Lys Lys Lys Thr Ala
        275
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<210> 166
<211> 278
<212> PRT
<213> Phaeodactylum tricornutum

<400> 166

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Met Met Val Pro Ser Ser Tyr Asp Glu Tyr Ile Val Met Val Asn Asp
1               5                  10                  15
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Leu Gly Asp Ser Ile Leu Ser Trp Ala Asp Pro Asp His Tyr Arg Gly
            20                  25                  30
His Thr Glu Gly Trp Glu Phe Thr Asp Phe Ser Ala Ala Phe Ser Ile
        35                  40                  45
Ala Val Ala Tyr Leu Leu Phe Val Phe Val Gly Ser Leu Ile Met Ser
    50                  55                  60
Met Gly Val Pro Ala Ile Asp Pro Tyr Pro Leu Lys Phe Val Tyr Asn
65                  70                  75                  80
Val Ser Gln Ile Met Leu Cys Ala Tyr Met Thr Ile Glu Ala Ser Leu
                85                  90                  95
Leu Ala Tyr Arg Asn Gly Tyr Thr Phe Trp Pro Cys Asn Asp Trp Asp
            100                 105                 110
Phe Glu Lys Pro Pro Ile Ala Lys Leu Leu Trp Leu Phe Tyr Val Ser
        115                 120                 125
Lys Ile Trp Asp Phe Trp Asp Thr Ile Phe Ile Val Leu Gly Lys Lys
    130                 135                 140
Trp Arg Gln Leu Ser Phe Leu His Val Tyr His His Thr Thr Ile Phe
145                 150                 155                 160
Leu Phe Tyr Trp Leu Asn Ala His Val Asn Phe Asp Gly Asp Ile Phe
                165                 170                 175
Leu Thr Ile Val Leu Asn Gly Phe Ile His Thr Val Met Tyr Thr Tyr
            180                 185                 190
Tyr Phe Ile Cys Met His Thr Lys Val Pro Glu Thr Gly Lys Ser Leu
        195                 200                 205
Pro Ile Trp Trp Lys Ser Ser Leu Thr Ser Met Gln Leu Val Gln Phe
    210                 215                 220
Ile Thr Met Met Thr Gln Ala Ile Met Ile Leu Tyr Lys Gly Cys Ala
225                 230                 235                 240
Ala Pro His Ser Arg Val Val Thr Ser Tyr Leu Val Tyr Ile Leu Ser
                245                 250                 255
Leu Phe Ile Leu Phe Ala Gln Phe Phe Val Ser Ser Tyr Leu Lys Pro
            260                 265                 270
Lys Lys Lys Lys Thr Ala
        275


<210> 167
<211> 272
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is any amino acid
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<222> (4)..(10)
<223> xaa in position 4 to 10 is any amino acid
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<222> (12)..(13)
<223> xaa in position 12 to 13 is any amino acid
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<222> (15)..(16)
<223> xaa in position 15 to 16 is any amino acid
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<222> (18)..(18)
<223> xaa in position 18 is any amino acid
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<222> (22)..(23)
<223> xaa in position 22 to 23 is any amino acid
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<222> (24)..(24)
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<223> xaa in position 24 is any or no amino acid
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<222> (26)..(28)
<223> xaa in position 26 to 28 is any amino acid
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<222> (30)..(30)
<223> xaa in position 30 is any amino acid
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<222> (32)..(34)
<223> xaa in position 32 to 34 is any amino acid
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<222> (37)..(38)
<223> xaa in position 37 to 38 is any amino acid
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<221> Variant
<222> (40)..(41)
<223> xaa in position 40 to 41 is any amino acid
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<221> Variant
<222> (44)..(45)
<223> xaa in position 44 to 45 is any amino acid
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<221> Variant
<222> (47)..(48)
<223> xaa in position 47 to 48 is any amino acid
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<221> Variant
<222> (51)..(52)
<223> xaa in position 51 to 52 is any amino acid
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<221> Variant
<222> (55)..(56)
<223> xaa in position 55 to 56 is any amino acid
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<221> Variant
<222> (58)..(60)
<223> xaa in position 58 to 60 is any amino acid
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<222> (61)..(61)
<223> xaa in position 61 is any or no amino acid
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<221> Variant
<222> (64)..(64)
<223> xaa in position 64 is any amino acid
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<221> Variant
<222> (69)..(69)
<223> xaa in position 69 is any amino acid
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<222> (72)..(72)
<223> xaa in position 72 is any amino acid
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<222> (79)..(79)
<223> xaa in position 79 is any amino acid
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<222> (86)..(86)
<223> xaa in position 86 is any amino acid
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<222> (89)..(90)

<223> xaa in position 89 to 90 is any amino acid
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<222> (99)..(100)
<223> xaa in position 99 to 100 is any amino acid
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<222> (104)..(109)
<223> xaa in position 104 to 109 is any amino acid
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<222> (112)..(112)
<223> xaa in position 112 is any amino acid
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<222> (114)..(114)
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<222> (124)..(124)
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<222> (162)..(162)
<223> xaa in position 162 is any amino acid
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<222> (164)..(165)
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<222> (174)..(174)
<223> xaa in position 174 is any amino acid
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<222> (195)..(197)
<223> xaa in position 195 to 197 is any amino acid
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<221> Variant
<222> (212)..(213)
<223> xaa in position 212 to 213 is any amino acid
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<222> (216)..(216)
<223> xaa in position 216 is any amino acid
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<222> (219)..(220)
<223> xaa in position 219 to 220 is any amino acid
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<222> (223)..(223)
<223> xaa in position 223 is any amino acid
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<222> (226)..(231)
<223> xaa in position 226 to 231 is any amino acid
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<222> (234)..(238)
<223> xaa in position 234 to 238 is any amino acid
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<222> (240)..(243)

<223> xaa in position 240 to 243 is any amino acid
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<222> (245)..(245)
<223> xaa in position 245 is any amino acid
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<222> (247)..(248)
<223> xaa in position 247 to 248 is any amino acid
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<222> (253)..(253)
<223> xaa in position 253 is any amino acid
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<222> (261)..(261)
<223> xaa in position 261 is any amino acid
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<222> (264)..(265)
<223> xaa in position 264 to 265 is any amino acid
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<222> (271)..(271)
<223> xaa in position 271 is any amino acid

<400> 167

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Asp xaa Tyr xaa xaa xaa xaa xaa xaa xaa Gly xaa xaa Ile xaa xaa
  1               5                  10                  15
Trp xaa Asp Pro Asp xaa xaa xaa Arg xaa xaa xaa Glu xaa Trp xaa
            20                  25                  30
xaa xaa Asp Phe xaa xaa Ala xaa xaa Ile Ala xaa xaa Tyr xaa xaa
        35                  40                  45
Phe Val xaa xaa Gly Ser xaa xaa Met xaa xaa xaa xaa Pro Ala xaa
    50                  55                  60
Asp Pro Tyr Pro xaa Lys Phe xaa Tyr Asn Val Ser Gln Ile xaa Leu
65                  70                  75                  80
Cys Ala Tyr Met Thr xaa Glu Ala xaa xaa Leu Ala Tyr Arg Asn Gly
                85                  90                  95
Tyr Thr xaa xaa Pro Cys Asn xaa xaa xaa xaa xaa xaa Pro Pro xaa
            100                 105                 110
Ala xaa Leu Leu Trp Leu Phe Tyr xaa Ser Lys xaa Trp Asp Phe Trp
        115                 120                 125
Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gln Leu Ser Phe
    130                 135                 140
Leu His Val Tyr His His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
145                 150                 155                 160
Ala xaa Val xaa xaa Asp Gly Asp Ile Phe Leu Thr Ile xaa Leu Asn
                165                 170                 175
Gly Phe Ile His Thr Val Met Tyr Thr Tyr Tyr Phe Ile Cys Met His
            180                 185                 190
Thr Lys xaa xaa xaa Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser
        195                 200                 205
Ser Leu Thr xaa xaa Gln Leu xaa Gln Phe xaa xaa Met Met xaa Gln
    210                 215                 220
Ala xaa xaa xaa xaa xaa xaa Gly Cys xaa xaa xaa xaa xaa Arg xaa
225                 230                 235                 240
xaa xaa xaa Tyr xaa Val xaa xaa Leu Ser Leu Phe xaa Leu Phe Ala
                245                 250                 255
Gln Phe Phe Val xaa Ser Tyr xaa xaa Pro Lys Lys Lys Lys xaa Ala
            260                 265                 270
```

<210> 168
<211> 59
<212> PRT
<213> Artificial sequence

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<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is any amino acid
<220>
<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is any amino acid
<220>
<221> Variant
<222> (30)..(34)
<223> xaa in position 30 to 34 is any or no amino acid
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Leu or Val

<400> 168
Gly Lys Lys Trp Arg Gln Leu Ser Phe Leu His Val Tyr His His Thr
  1               5                  10                  15
Thr Ile Phe Leu Phe Tyr Trp Leu Asn Ala xaa Val xaa xaa xaa xaa
            20                  25                  30
xaa xaa Phe Leu Thr Ile xaa Leu Asn Gly Phe Ile His Thr Val Met
        35                  40                  45
Tyr Thr Tyr Tyr Phe Ile Cys Met His Thr Lys
    50                  55


<210> 169
<211> 60
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Phe or Met
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is Ile or Val
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any or no amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is any or no amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Phe or Val
<220>
<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is any amino acid
<220>
<221> Variant
<222> (33)..(33)
<223> xaa in position 33 is Asp or His
<220>
<221> Variant
```

<222> (34)..(34)
<223> xaa in position 34 is Phe or Trp
<220>
<221> Variant
<222> (35)..(35)
<223> xaa in position 35 is Asp or Asn
<220>
<221> Variant
<222> (36)..(36)
<223> xaa in position 36 is Phe or Val
<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is Glu or Asn
<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is Asp or Lys
<220>
<221> Variant
<222> (41)..(41)
<223> xaa in position 41 is Ile or Val
<220>
<221> Variant
<222> (43)..(43)
<223> xaa in position 43 is any amino acid
<220>
<221> Variant
<222> (50)..(50)
<223> xaa in position 50 is Ile or Val
<220>
<221> Variant
<222> (53)..(53)
<223> xaa in position 53 is Ile or Val

<400> 169
Tyr Asn Val Ser Gln Ile xaa Leu Cys Ala Tyr Met Thr xaa Glu Ala
1 5 10 15
xaa xaa Leu xaa Ala Tyr Arg Asn Gly Tyr Thr xaa xaa Pro Cys Asn
20 25 30
xaa xaa xaa xaa xaa xaa Pro Pro xaa Ala xaa Leu Leu Trp Leu Phe
35 40 45
Tyr xaa Ser Lys xaa Trp Asp Phe Trp Asp Thr Ile
50 55 60

<210> 170
<211> 39
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is Ala or Ser
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Phe or Met
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Leu or Val
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any or no amino acid
<220>

<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is any amino acid
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is any amino acid
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is any or no amino acid
<220>
<221> Variant
<222> (26)..(27)
<223> xaa in position 26 to 27 is any amino acid
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Ile or Leu
<220>
<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is Leu or Val
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Phe or Tyr
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is His or Lys
<220>
<221> Variant
<222> (34)..(34)
<223> xaa in position 34 is Ala or Asp
<220>
<221> Variant
<222> (35)..(35)
<223> xaa in position 35 is any amino acid
<220>
<221> Variant
<222> (36)..(36)
<223> xaa in position 36 is Pro or Val
<220>
<221> Variant
<222> (37)..(38)
<223> xaa in position 37 to 38 is any amino acid

<400> 170
Leu Pro Ile Trp Trp Lys Ser Ser Leu Thr xaa xaa Gln Leu xaa Gln
  1   5   10   15
Phe xaa Ile xaa Met xaa xaa Gln Ala xaa xaa xaa xaa xaa xaa Gly
            20   25   30
Cys xaa xaa xaa xaa xaa Arg
        35

<210> 171
<211> 831
<212> DNA
<213> Traustochytrium sp.

<220>
<221> CDS
<222> (1)..(831)

<400> 171
atg gac gtc gtc gag cag caa tgg cgc cgc ttc gtg gac gcc gtg gac 48
Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp

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1                   5                  10                  15
aac gga atc gtg gag ttc atg gag cat gag aag ccc aac aag ctg aac        96
Asn Gly Ile Val Glu Phe Met Glu His Glu Lys Pro Asn Lys Leu Asn
            20                  25                  30
gag ggc aag ctc ttc acc tcg acc gag gag atg atg gcg ctt atc gtc       144
Glu Gly Lys Leu Phe Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
ggc tac ctg gcg ttc gtg gtc ctc ggg tcc gcc ttc atg aag gcc ttt       192
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
gtc gat aag cct ttc gag ctc aag ttc ctc aag ctc gtg cac aac atc       240
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
ttc ctc acc ggt ctg tcc atg tac atg gcc acc gag tgc gcg cgc cag       288
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
gca tac ctc ggc ggc tac aag ctc ttt ggc aac ccg atg gag aag ggc       336
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
acc gag tcg cac gcc ccg ggc atg gcc aac atc atc tac atc ttc tac       384
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
gtg agc aag ttc ctc gaa ttc ctc gac acc gtc ttc atg atc ctc ggc       432
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
aag aag tgg aag cag ctc agc ttt ctc cac gtc tac cac cac gcg agc       480
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
atc agc ttc atc tgg ggc atc atc gcc cgc ttc gcg ccc ggt ggc gac       528
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
gcc tac ttc tct acc atc ctc aac agc agc gtg cat gtc gtg ctc tac       576
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
ggc tac tac gcc tcg acc acc ctc ggc tac acc ttc atg cgc ccg ctg       624
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
cgc ccg tac att acc acc att cag ctc acg cag ttc atg gcc atg gtc       672
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
gtc cag tcc gtc tat gac tac tac aac ccc tgc gac tac ccg cag ccc       720
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
ctc gtc aag ctg ctc ttc tgg tac atg ctc acc atg ctc ggc ctc ttc       768
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
ggc aac ttc ttc gtg cag cag tac ctc aag ccc aag gcg ccc aag aag       816
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
cag aag acc atc taa                                               831
Gln Lys Thr Ile
        275
```

<210> 172
<211> 276
<212> PRT
<213> Traustochytrium sp.

<400> 172

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Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                  10                  15
Asn Gly Ile Val Glu Phe Met Glu His Glu Lys Pro Asn Lys Leu Asn
            20                  25                  30
Glu Gly Lys Leu Phe Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
```

```
65                  70                  75                  80
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
Gln Lys Thr Ile
        275
```

<210> 173
<211> 1047
<212> DNA
<213> Marchantia polymorpha

<220>
<221> CDS
<222> (1)..(1047)

<400> 173

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atg gcg acg aag agc ggg agc gga ttg ctc gag tgg ata gca gta gcg        48
Met Ala Thr Lys Ser Gly Ser Gly Leu Leu Glu Trp Ile Ala Val Ala
1               5                  10                  15
gcg aag atg aag caa gct cgg agc agc ccc gag ggt gag atc gtg ggt        96
Ala Lys Met Lys Gln Ala Arg Ser Ser Pro Glu Gly Glu Ile Val Gly
            20                  25                  30
ggg aat agg atg ggc tct gga aac gga gct gag tgg acc acg agt ctg       144
Gly Asn Arg Met Gly Ser Gly Asn Gly Ala Glu Trp Thr Thr Ser Leu
        35                  40                  45
att cat gca ttt ttg aat gcc acg aat ggg aag agc ggc ggt gct tcg       192
Ile His Ala Phe Leu Asn Ala Thr Asn Gly Lys Ser Gly Gly Ala Ser
    50                  55                  60
aaa gtg agg cct ctc gag gag aga atc ggg gag gcg gtg ttc aga gtt       240
Lys Val Arg Pro Leu Glu Glu Arg Ile Gly Glu Ala Val Phe Arg Val
65                  70                  75                  80
ctt gaa gat gtc gtg ggc gtg gat att agg aag ccg aat cct gtc acg       288
Leu Glu Asp Val Val Gly Val Asp Ile Arg Lys Pro Asn Pro Val Thr
                85                  90                  95
aag gac ctt ccg atg gtc gag agt ccc gtg ccc gtg ttg gcc tgc att       336
Lys Asp Leu Pro Met Val Glu Ser Pro Val Pro Val Leu Ala Cys Ile
            100                 105                 110
tct ctg tac ttg ctc gtg gtg tgg ctt tgg tct tct cac att aag gcg       384
Ser Leu Tyr Leu Leu Val Val Trp Leu Trp Ser Ser His Ile Lys Ala
        115                 120                 125
tct ggc caa aag ccc agg aag gag gac ccg ctc gcc ctg cgt tgc ctt       432
Ser Gly Gln Lys Pro Arg Lys Glu Asp Pro Leu Ala Leu Arg Cys Leu
    130                 135                 140
gtg att gcc cac aat ctg ttc ctg tgt tgc ttg agc ttg ttc atg tgc       480
Val Ile Ala His Asn Leu Phe Leu Cys Cys Leu Ser Leu Phe Met Cys
145                 150                 155                 160
```

```
gtc ggt ctc att gcc gca gct cga cat tac ggg tat agt gta tgg ggg          528
Val Gly Leu Ile Ala Ala Ala Arg His Tyr Gly Tyr Ser Val Trp Gly
                165                 170                 175
aac tac tac aga gaa aga gaa ccc gca atg aat ttg ctc att tac gtg          576
Asn Tyr Tyr Arg Glu Arg Glu Pro Ala Met Asn Leu Leu Ile Tyr Val
            180                 185                 190
ttc tac atg tcg aag ctg tac gaa ttt atg gac acg gcc att atg tta          624
Phe Tyr Met Ser Lys Leu Tyr Glu Phe Met Asp Thr Ala Ile Met Leu
        195                 200                 205
ttc aga aga aat ctg cga caa gtc acg tac ttg cat gta tat cac cac          672
Phe Arg Arg Asn Leu Arg Gln Val Thr Tyr Leu His Val Tyr His His
    210                 215                 220
gca agc atc gca atg att tgg tgg ata att tgc tat cgg ttt cca gga          720
Ala Ser Ile Ala Met Ile Trp Trp Ile Ile Cys Tyr Arg Phe Pro Gly
225                 230                 235                 240
gct gat tcg tat ttc tcc gca gca ttc aat tcc tgt atc cat gta gcg          768
Ala Asp Ser Tyr Phe Ser Ala Ala Phe Asn Ser Cys Ile His Val Ala
                245                 250                 255
atg tac ctg tat tat cta ctc gcg gca acc gtc gcc aga gac gaa aag          816
Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Val Ala Arg Asp Glu Lys
            260                 265                 270
cgg aga cgc aaa tat ctc ttc tgg gga aag tat ctg acc atc ata caa          864
Arg Arg Arg Lys Tyr Leu Phe Trp Gly Lys Tyr Leu Thr Ile Ile Gln
        275                 280                 285
atg ctt cag ttt ttg tcc ttc att ggg cag gcg att tat gca atg tgg          912
Met Leu Gln Phe Leu Ser Phe Ile Gly Gln Ala Ile Tyr Ala Met Trp
    290                 295                 300
aag ttt gaa tac tat ccc aag ggc ttt ggc agg atg ttg ttc ttt tac          960
Lys Phe Glu Tyr Tyr Pro Lys Gly Phe Gly Arg Met Leu Phe Phe Tyr
305                 310                 315                 320
tct gta tca ttg ttg gca ttt ttc ggc aac ttc ttt gtc aaa aag tat         1008
Ser Val Ser Leu Leu Ala Phe Phe Gly Asn Phe Phe Val Lys Lys Tyr
                325                 330                 335
tcg aac gct tca cag cct aag aca gtt aaa gtg gag tga                     1047
Ser Asn Ala Ser Gln Pro Lys Thr Val Lys Val Glu
            340                 345
```

<210> 174
<211> 348
<212> PRT
<213> Marchantia polymorpha

<400> 174

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Met Ala Thr Lys Ser Gly Ser Gly Leu Leu Glu Trp Ile Ala Val Ala
1               5                  10                  15
Ala Lys Met Lys Gln Ala Arg Ser Ser Pro Glu Gly Glu Ile Val Gly
            20                  25                  30
Gly Asn Arg Met Gly Ser Gly Asn Gly Ala Glu Trp Thr Thr Ser Leu
        35                  40                  45
Ile His Ala Phe Leu Asn Ala Thr Asn Gly Lys Ser Gly Gly Ala Ser
    50                  55                  60
Lys Val Arg Pro Leu Glu Glu Arg Ile Gly Glu Ala Val Phe Arg Val
65                  70                  75                  80
Leu Glu Asp Val Val Gly Val Asp Ile Arg Lys Pro Asn Pro Val Thr
                85                  90                  95
Lys Asp Leu Pro Met Val Glu Ser Pro Val Pro Val Leu Ala Cys Ile
            100                 105                 110
Ser Leu Tyr Leu Leu Val Val Trp Leu Trp Ser Ser His Ile Lys Ala
        115                 120                 125
Ser Gly Gln Lys Pro Arg Lys Glu Asp Pro Leu Ala Leu Arg Cys Leu
    130                 135                 140
Val Ile Ala His Asn Leu Phe Leu Cys Cys Leu Ser Leu Phe Met Cys
145                 150                 155                 160
Val Gly Leu Ile Ala Ala Ala Arg His Tyr Gly Tyr Ser Val Trp Gly
                165                 170                 175
Asn Tyr Tyr Arg Glu Arg Glu Pro Ala Met Asn Leu Leu Ile Tyr Val
            180                 185                 190
Phe Tyr Met Ser Lys Leu Tyr Glu Phe Met Asp Thr Ala Ile Met Leu
```

```
          195                 200                 205
Phe Arg Arg Asn Leu Arg Gln Val Thr Tyr Leu His Val Tyr His His
    210                 215                 220
Ala Ser Ile Ala Met Ile Trp Trp Ile Ile Cys Tyr Arg Phe Pro Gly
225                 230                 235                 240
Ala Asp Ser Tyr Phe Ser Ala Ala Phe Asn Ser Cys Ile His Val Ala
                245                 250                 255
Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Val Ala Arg Asp Glu Lys
            260                 265                 270
Arg Arg Arg Lys Tyr Leu Phe Trp Gly Lys Tyr Leu Thr Ile Ile Gln
        275                 280                 285
Met Leu Gln Phe Leu Ser Phe Ile Gly Gln Ala Ile Tyr Ala Met Trp
    290                 295                 300
Lys Phe Glu Tyr Tyr Pro Lys Gly Phe Gly Arg Met Leu Phe Phe Tyr
305                 310                 315                 320
Ser Val Ser Leu Leu Ala Phe Phe Gly Asn Phe Phe Val Lys Lys Tyr
                325                 330                 335
Ser Asn Ala Ser Gln Pro Lys Thr Val Lys Val Glu
            340                 345
```

<210> 175
<211> 831
<212> DNA
<213> Thraustochytrium sp

<220>
<221> CDS
<222> (1)..(831)

<400> 175

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atg gat gtc gtc gag cag caa tgg cgc cgc ttc gtg gac gcc gtg gac      48
Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                  10                  15
aac gga atc gtg gag ttc atg gag cat gag gag ccc aac aag ctg aac      96
Asn Gly Ile Val Glu Phe Met Glu His Glu Glu Pro Asn Lys Leu Asn
            20                  25                  30
gag ggc aag ctc tcc acc tcg acc gag gag atg atg gcg ctt atc gtc     144
Glu Gly Lys Leu Ser Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
ggc tac ctg gcg ttc gtg gtc ctc ggg tcc gcc ttc atg aag gcc ttt     192
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
gtc gat aag cct ttc gag ctc aag ttc ctc aag ctc gtg cac aac atc     240
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
ttc ctc acc ggt ctg tcc atg tac atg gcc acc gag tgc gcg cgc cag     288
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
gca tac ctc ggc ggc tac aag ctc ttt ggc aac ccg atg gag aag ggc     336
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
acc gag tcg cac gcc ccg ggc atg gcc aac atc atc tac atc ttc tac     384
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
gtg agc aag ttc ctc gaa ttc ctc gac acc gtc ttc atg atc ctc ggc     432
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
aag aag tgg aag cag ctc agc ttt ctc cac gtc tac cac cac gcg agc     480
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
atc agc ttc atc tgg ggc atc atc gcc cgc ttc gcg ccc ggt ggc gac     528
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
gcc tac ttc tct acc atc ctc aac agc agc gtg cat gtc gtg ctc tac     576
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
ggc tac tac gcc tcg acc acc ctc ggc tac acc ttc atg cgc ccg ctg     624
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
```

```
          195                 200                 205
cgc ccg tac att acc acc att cag ctc acg cag ttc atg gcc atg gtc        672
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
gtc cag tcc gtc tat gac tac tac aac ccc tgc gac tac ccg cag ccc        720
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
ctc gtc aag ctg ctc ttc tgg tac atg ctc acc atg ctc ggc ctc ttc        768
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
ggc aac ttc ttc gtg cag cag tac ctc aag ccc aag gcg ccc aag aag        816
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
cag aag acc atc taa                                                   831
Gln Lys Thr Ile
        275

<210> 176
<211> 276
<212> PRT
<213> Thraustochytrium sp

<400> 176
Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
1               5                  10                  15
Asn Gly Ile Val Glu Phe Met Glu His Glu Glu Pro Asn Lys Leu Asn
            20                  25                  30
Glu Gly Lys Leu Ser Thr Ser Thr Glu Glu Met Met Ala Leu Ile Val
        35                  40                  45
Gly Tyr Leu Ala Phe Val Val Leu Gly Ser Ala Phe Met Lys Ala Phe
    50                  55                  60
Val Asp Lys Pro Phe Glu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65                  70                  75                  80
Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Glu Cys Ala Arg Gln
                85                  90                  95
Ala Tyr Leu Gly Gly Tyr Lys Leu Phe Gly Asn Pro Met Glu Lys Gly
            100                 105                 110
Thr Glu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
        115                 120                 125
Val Ser Lys Phe Leu Glu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
    130                 135                 140
Lys Lys Trp Lys Gln Leu Ser Phe Leu His Val Tyr His His Ala Ser
145                 150                 155                 160
Ile Ser Phe Ile Trp Gly Ile Ile Ala Arg Phe Ala Pro Gly Gly Asp
                165                 170                 175
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
            180                 185                 190
Gly Tyr Tyr Ala Ser Thr Thr Leu Gly Tyr Thr Phe Met Arg Pro Leu
        195                 200                 205
Arg Pro Tyr Ile Thr Thr Ile Gln Leu Thr Gln Phe Met Ala Met Val
    210                 215                 220
Val Gln Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gln Pro
225                 230                 235                 240
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gly Leu Phe
                245                 250                 255
Gly Asn Phe Phe Val Gln Gln Tyr Leu Lys Pro Lys Ala Pro Lys Lys
            260                 265                 270
Gln Lys Thr Ile
        275

<210> 177
<211> 879
<212> DNA
<213> Ostreococcus tauri

<220>
<221> CDS
<222> (1)..(879)
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<400> 177

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atg agt ggc tta cgt gca ccc aac ttt tta cac aga ttc tgg aca aag        48
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
tgg gac tac gcg att tcc aaa gtc gtc ttc acg tgt gcc gac agt ttt        96
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
            20                  25                  30
cag tgg gac atc ggg cca gtg agt tcg agt acg gcg cat tta ccc gcc       144
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
        35                  40                  45
att gaa tcc cct acc cca ctg gtg act agc ctc ttg ttc tac tta gtc       192
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
    50                  55                  60
aca gtt ttc ttg tgg tat ggt cgt tta acc agg agt tca gac aag aaa       240
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
att aga gag cct acg tgg tta aga aga ttc ata ata tgt cat aat gcg       288
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
ttc ttg ata gtc ctc agt ctt tac atg tgc ctt ggt tgt gtg gcc caa       336
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
gcg tat cag aat gga tat act tta tgg ggt aat gaa ttc aag gcc acg       384
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
gaa act cag ctt gct ctc tac att tac att ttt tac gta agt aaa ata       432
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
tac gag ttt gta gat act tac att atg ctt ctc aag aat aac ttg cgg       480
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
caa gta agt ttc cta cac att tat cac cac agc acg att tcc ttt att       528
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
tgg tgg atc att gct cgg agg gct ccg ggt ggt gat gct tac ttc agc       576
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
gcg gcc ttg aac tca tgg gta cac gtg tgc atg tac acc tat tat cta       624
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
tta tca acc ctt att gga aaa gaa gat cct aag cgt tcc aac tac ctt       672
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
tgg tgg ggt cgc cac cta acg caa atg cag atg ctt cag ttt ttc ttc       720
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
aac gta ctt caa gcg ttg tac tgc gct tcg ttc tct acg tat ccc aag       768
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
ttt ttg tcc aaa att ctg ctc gtc tat atg atg agc ctt ctc ggc ttg       816
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
ttt ggg cat ttc tac tat tcc aag cac ata gca gca gct aag ctc cag       864
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
aaa aaa cag cag tga                                                   879
Lys Lys Gln Gln
    290
```

<210> 178
<211> 292
<212> PRT
<213> Ostreococcus tauri

<400> 178

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Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1               5                  10                  15
```

```
Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
                20                  25                  30
Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
            35                  40                  45
Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
        50                  55                  60
Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65                  70                  75                  80
Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
                85                  90                  95
Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
            100                 105                 110
Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
        115                 120                 125
Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
    130                 135                 140
Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145                 150                 155                 160
Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
                165                 170                 175
Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
            180                 185                 190
Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
        195                 200                 205
Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
    210                 215                 220
Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225                 230                 235                 240
Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
                245                 250                 255
Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
            260                 265                 270
Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln
        275                 280                 285
Lys Lys Gln Gln
    290
```

<210> 179
<211> 873
<212> DNA
<213> Marchantia polymorpha

<220>
<221> CDS
<222> (1)..(873)

<400> 179

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atg gag gcg tac gag atg gtg gat agt ttt gtg tcg aag acg gtt ttc        48
Met Glu Ala Tyr Glu Met Val Asp Ser Phe Val Ser Lys Thr Val Phe
1               5                   10                  15
gaa acg ctg cag aga ctg agg ggc gga gtc gtg ttg acg gaa tct gcg        96
Glu Thr Leu Gln Arg Leu Arg Gly Gly Val Val Leu Thr Glu Ser Ala
            20                  25                  30
atc acc aaa ggt ttg cca tgc gtc gat agc ccg acg ccg atc gtt ctt       144
Ile Thr Lys Gly Leu Pro Cys Val Asp Ser Pro Thr Pro Ile Val Leu
        35                  40                  45
ggg ttg tcg tcc tac ttg aca ttc gtg ttt ctc ggg ctc att gtc atc       192
Gly Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gly Leu Ile Val Ile
    50                  55                  60
aag agc ctg gat ctt aag ccc cgc tcc aag gag ccc gcc att ttg aac       240
Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Glu Pro Ala Ile Leu Asn
65                  70                  75                  80
ctg ttt gtg atc ttc cac aac ttc gtc tgc ttc gca ctc agt ctg tac       288
Leu Phe Val Ile Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr
                85                  90                  95
atg tgc gtg gga att gtc cgt caa gct atc ctc aac agg tac tct ctg       336
Met Cys Val Gly Ile Val Arg Gln Ala Ile Leu Asn Arg Tyr Ser Leu
            100                 105                 110
```

```
tgg ggc aat gcg tac aat ccc aaa gaa gtt caa atg ggc cac ctg ctc        384
Trp Gly Asn Ala Tyr Asn Pro Lys Glu Val Gln Met Gly His Leu Leu
        115                 120                 125
tac att ttc tac atg tca aag tac atc gag ttt atg gac acg gtc att        432
Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Glu Phe Met Asp Thr Val Ile
    130                 135                 140
atg att ttg aag cgc aac acg cgc cag atc act gtg ttg cat gtg tac        480
Met Ile Leu Lys Arg Asn Thr Arg Gln Ile Thr Val Leu His Val Tyr
145                 150                 155                 160
cac cac gca tcc atc tcc ttc atc tgg tgg atc atc gcc tac cat gct        528
His His Ala Ser Ile Ser Phe Ile Trp Trp Ile Ile Ala Tyr His Ala
                165                 170                 175
cct ggc ggt gaa gct tat ttc tct gcc gca ttg aac tcc gga gta cat        576
Pro Gly Gly Glu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
            180                 185                 190
gtg ctc atg tac ctc tac tac ctt ttg gca gca act ctg gga aag aac        624
Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gly Lys Asn
        195                 200                 205
gag aaa gct cgc cgc aag tac cta tgg tgg gga aaa tac ttg aca cag        672
Glu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr Gln
    210                 215                 220
ctg cag atg ttc cag ttt gtc ctt aac atg att cag gct tac tac gat        720
Leu Gln Met Phe Gln Phe Val Leu Asn Met Ile Gln Ala Tyr Tyr Asp
225                 230                 235                 240
att aag aac aac tcg cct tac cca caa ttt ttg atc cag att ttg ttc        768
Ile Lys Asn Asn Ser Pro Tyr Pro Gln Phe Leu Ile Gln Ile Leu Phe
                245                 250                 255
tac tac atg atc tcg ctt tta gcg cta ttt gga aac ttt tac gtt cac        816
Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val His
            260                 265                 270
aaa tac gta tca gcg ccc gca aaa cct gcg aag atc aag agc aaa aag        864
Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
        275                 280                 285
gca gaa taa                                                           873
Ala Glu
    290
```

<210> 180
<211> 290
<212> PRT
<213> Marchantia polymorpha

<400> 180

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Met Glu Ala Tyr Glu Met Val Asp Ser Phe Val Ser Lys Thr Val Phe
1               5                  10                  15
Glu Thr Leu Gln Arg Leu Arg Gly Gly Val Val Leu Thr Glu Ser Ala
            20                  25                  30
Ile Thr Lys Gly Leu Pro Cys Val Asp Ser Pro Thr Pro Ile Val Leu
        35                  40                  45
Gly Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gly Leu Ile Val Ile
    50                  55                  60
Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Glu Pro Ala Ile Leu Asn
65                  70                  75                  80
Leu Phe Val Ile Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr
                85                  90                  95
Met Cys Val Gly Ile Val Arg Gln Ala Ile Leu Asn Arg Tyr Ser Leu
            100                 105                 110
Trp Gly Asn Ala Tyr Asn Pro Lys Glu Val Gln Met Gly His Leu Leu
        115                 120                 125
Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Glu Phe Met Asp Thr Val Ile
    130                 135                 140
Met Ile Leu Lys Arg Asn Thr Arg Gln Ile Thr Val Leu His Val Tyr
145                 150                 155                 160
His His Ala Ser Ile Ser Phe Ile Trp Trp Ile Ile Ala Tyr His Ala
                165                 170                 175
Pro Gly Gly Glu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
            180                 185                 190
Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gly Lys Asn
```

```
          195                 200                 205
Glu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr Gln
    210                 215                 220
Leu Gln Met Phe Gln Phe Val Leu Asn Met Ile Gln Ala Tyr Tyr Asp
225                 230                 235                 240
Ile Lys Asn Asn Ser Pro Tyr Pro Gln Phe Leu Ile Gln Ile Leu Phe
                245                 250                 255
Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val His
            260                 265                 270
Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
        275                 280                 285
Ala Glu
    290

<210> 181
<211> 873
<212> DNA
<213> Physcomitrella patens

<220>
<221> CDS
<222> (1)..(873)

<400> 181
atg gag gtc gtg gag aga ttc tac ggt gag ttg gat ggg aag gtc tcg        48
Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
1               5                  10                  15
cag ggc gtg aat gca ttg ctg ggt agt ttt ggg gtg gag ttg acg gat        96
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30
acg ccc act acc aaa ggc ttg ccc ctc gtt gac agt ccc aca ccc atc       144
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45
gtc ctc ggt gtt tct gta tac ttg act att gtc att gga ggg ctt ttg       192
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60
tgg ata aag gcc agg gat ctg aaa ccg cgc gcc tcg gag cca ttt ttg       240
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80
ctc caa gct ttg gtg ctt gtg cac aac ctg ttc tgt ttt gcg ctc agt       288
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95
ctg tat atg tgc gtg ggc atc gct tat cag gct att acc tgg cgg tac       336
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110
tct ctc tgg ggc aat gca tac aat cct aaa cat aaa gag atg gcg att       384
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125
ctg gta tac ttg ttc tac atg tct aag tac gtg gaa ttc atg gat acc       432
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140
gtt atc atg ata ctg aag cgc agc acc agg caa ata agc ttc ctc cac       480
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160
gtt tat cat cat tct tca att tcc ctc att tgg tgg gct att gct cat       528
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175
cac gct cct ggc ggt gaa gca tat tgg tct gcg gct ctg aac tca gga       576
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190
gtg cat gtt ctc atg tat gcg tat tac ttc ttg gct gcc tgc ctt cga       624
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205
agt agc cca aag tta aaa aat aag tac ctt ttt tgg ggc agg tac ttg       672
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220
aca caa ttc caa atg ttc cag ttt atg ctg aac tta gtg cag gct tac       720
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
```

```
225                 230                 235                 240
tac gac atg aaa acg aat gcg cca tat cca caa tgg ctg atc aag att      768
Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255
ttg ttc tac tac atg atc tcg ttg ctg ttt ctt ttc ggc aat ttt tac      816
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
            260                 265                 270
gta caa aaa tac atc aaa ccc tct gac gga aag caa aag gga gct aaa      864
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
        275                 280                 285
act gag tga                                                         873
Thr Glu
    290
```

<210> 182
<211> 290
<212> PRT
<213> Physcomitrella patens

<400> 182

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Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
1               5                  10                  15
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240
Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
            260                 265                 270
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
        275                 280                 285
Thr Glu
    290
```

<210> 183
<211> 957
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(957)

<400> 183

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atg gag tcg att gcg cca ttc ctc cca tca aag atg ccg caa gat ctg        48
Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
1               5                  10                  15
ttt atg gac ctt gcc acc gct atc ggt gtc cgg gcc gcg ccc tat gtc        96
Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
            20                  25                  30
gat cct ctc gag gcc gcg ctg gtg gcc cag gcc gag aag tac atc ccc       144
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
        35                  40                  45
acg att gtc cat cac acg cgt ggg ttc ctg gtc gcg gtg gag tcg cct       192
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
    50                  55                  60
ttg gcc cgt gag ctg ccg ttg atg aac ccg ttc cac gtg ctg ttg atc       240
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65                  70                  75                  80
gtg ctc gct tat ttg gtc acg gtc ttt gtg ggc atg cag atc atg aag       288
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
                85                  90                  95
aac ttt gag cgg ttc gag gtc aag acg ttt tcg ctc ctg cac aac ttt       336
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
            100                 105                 110
tgt ctg gtc tcg atc agc gcc tac atg tgc ggt ggg atc ctg tac gag       384
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
        115                 120                 125
gct tat cag gcc aac tat gga ctg ttt gag aac gct gct gat cat acc       432
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
    130                 135                 140
ttc aag ggt ctt cct atg gcc aag atg atc tgg ctc ttc tac ttc tcc       480
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145                 150                 155                 160
aag atc atg gag ttt gtc gac acc atg atc atg gtc ctc aag aag aac       528
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
                165                 170                 175
aac cgc cag atc tcc ttc ttg cac gtt tac cac cac agc tcc atc ttc       576
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
            180                 185                 190
acc atc tgg tgg ttg gtc acc ttt gtt gca ccc aac ggt gaa gcc tac       624
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
        195                 200                 205
ttc tct gct gcg ttg aac tcg ttc atc cat gtg atc atg tac ggc tac       672
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
    210                 215                 220
tac ttc ttg tcg gcc ttg ggc ttc aag cag gtg tcg ttc atc aag ttc       720
Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225                 230                 235                 240
tac atc acg cgc tcg cag atg aca cag ttc tgc atg atg tcg gtc cag       768
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
                245                 250                 255
tct tcc tgg gac atg tac gcc atg aag gtc ctt ggc cgc ccc gga tac       816
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
            260                 265                 270
ccc ttc ttc atc acg gct ctg ctt tgg ttc tac atg tgg acc atg ctc       864
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
        275                 280                 285
ggt ctc ttc tac aac ttt tac aga aag aac gcc aag ttg gcc aag cag       912
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
    290                 295                 300
gcc aag gcc gac gct gcc aag gag aag gca agg aag ttg cag taa           957
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305                 310                 315
```

<210> 184
<211> 318
<212> PRT
<213> Mortierella alpina

<400> 184

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Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
1               5                  10                  15
Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
            20                  25                  30
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
        35                  40                  45
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
    50                  55                  60
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65                  70                  75                  80
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
                85                  90                  95
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
            100                 105                 110
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
        115                 120                 125
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
    130                 135                 140
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145                 150                 155                 160
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
                165                 170                 175
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
            180                 185                 190
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
        195                 200                 205
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
    210                 215                 220
Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225                 230                 235                 240
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
                245                 250                 255
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
            260                 265                 270
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
        275                 280                 285
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
    290                 295                 300
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305                 310                 315
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<210> 185
<211> 251
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(11)
<223> xaa in position 2 to 11 is any amino acid
<220>
<221> Variant
<222> (14)..(15)
<223> xaa in position 14 to 15 is any amino acid
<220>
<221> Variant
<222> (17)..(23)
<223> xaa in position 17 to 23 is any amino acid
<220>
<221> Variant
<222> (25)..(25)
<223> xaa in position 25 is any amino acid
<220>
<221> Variant
<222> (26)..(28)
<223> xaa in position 26 to 28 is any or no amino acid
<220>

<221> Variant
<222> (30)..(31)
<223> xaa in position 30 to 31 is any amino acid
<220>
<221> Variant
<222> (32)..(34)
<223> xaa in position 32 to 34 is any or no amino acid
<220>
<221> Variant
<222> (36)..(37)
<223> xaa in position 36 to 37 is any amino acid
<220>
<221> Variant
<222> (39)..(44)
<223> xaa in position 39 to 44 is any amino acid
<220>
<221> Variant
<222> (47)..(51)
<223> xaa in position 47 to 51 is any amino acid
<220>
<221> Variant
<222> (54)..(54)
<223> xaa in position 54 is any amino acid
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<222> (57)..(63)
<223> xaa in position 57 to 63 is any amino acid
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<221> Variant
<222> (65)..(68)
<223> xaa in position 65 to 68 is any amino acid
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<221> Variant
<222> (70)..(70)
<223> xaa in position 70 is any amino acid
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<221> Variant
<222> (72)..(72)
<223> xaa in position 72 is any amino acid
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<221> Variant
<222> (75)..(82)
<223> xaa in position 75 to 82 is any amino acid
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<221> Variant
<222> (83)..(86)
<223> xaa in position 83 to 86 is any or no amino acid
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<221> Variant
<222> (88)..(91)
<223> xaa in position 88 to 91 is any amino acid
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<221> Variant
<222> (93)..(93)
<223> xaa in position 93 is any amino acid
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<221> Variant
<222> (96)..(96)
<223> xaa in position 96 is any amino acid
<220>
<221> Variant
<222> (99)..(100)
<223> xaa in position 99 to 100 is any amino acid
<220>
<221> Variant
<222> (103)..(103)
<223> xaa in position 103 is any amino acid
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<221> Variant
<222> (106)..(107)
<223> xaa in position 106 to 107 is any amino acid
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<221> Variant
<222> (109)..(109)
<223> xaa in position 109 is any amino acid
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<221> Variant
<222> (111)..(115)
<223> xaa in position 111 to 115 is any amino acid
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<222> (117)..(119)
<223> xaa in position 117 to 119 is any amino acid
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<221> Variant
<222> (126)..(126)
<223> xaa in position 126 is any amino acid
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<221> Variant
<222> (129)..(130)
<223> xaa in position 129 to 130 is any amino acid
<220>
<221> Variant
<222> (133)..(134)
<223> xaa in position 133 to 134 is any amino acid
<220>
<221> Variant
<222> (136)..(138)
<223> xaa in position 136 to 138 is any amino acid
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<221> Variant
<222> (143)..(143)
<223> xaa in position 143 is any amino acid
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<222> (148)..(149)
<223> xaa in position 148 to 149 is any amino acid
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<221> Variant
<222> (153)..(154)
<223> xaa in position 153 to 154 is any amino acid
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<222> (157)..(158)
<223> xaa in position 157 to 158 is any amino acid
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<222> (160)..(160)
<223> xaa in position 160 is any amino acid
<220>
<221> Variant
<222> (163)..(179)
<223> xaa in position 163 to 179 is any amino acid
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<221> Variant
<222> (180)..(185)
<223> xaa in position 180 to 185 is any or no amino acid
<220>
<221> Variant
<222> (187)..(188)
<223> xaa in position 187 to 188 is any amino acid
<220>
<221> Variant
<222> (190)..(191)
<223> xaa in position 190 to 191 is any amino acid
<220>

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<221> Variant
<222> (194)..(198)
<223> xaa in position 194 to 198 is any amino acid
<220>
<221> Variant
<222> (200)..(201)
<223> xaa in position 200 to 201 is any amino acid
<220>
<221> Variant
<222> (203)..(208)
<223> xaa in position 203 to 208 is any amino acid
<220>
<221> Variant
<222> (209)..(209)
<223> xaa in position 209 is any or no amino acid
<220>
<221> Variant
<222> (212)..(217)
<223> xaa in position 212 to 217 is any amino acid
<220>
<221> Variant
<222> (219)..(220)
<223> xaa in position 219 to 220 is any amino acid
<220>
<221> Variant
<222> (223)..(225)
<223> xaa in position 223 to 225 is any amino acid
<220>
<221> Variant
<222> (227)..(227)
<223> xaa in position 227 is any amino acid
<220>
<221> Variant
<222> (233)..(246)
<223> xaa in position 233 to 246 is any amino acid
<220>
<221> Variant
<222> (247)..(250)
<223> xaa in position 247 to 250 is any or no amino acid

<400> 185
Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Leu Xaa Xaa Val
  1               5                  10                  15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa
            20                  25                  30
Xaa Xaa Pro Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa His Asn Xaa Xaa
        35                  40                  45
Xaa Xaa Xaa Leu Ser Xaa Tyr Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala
    50                  55                  60
Xaa Xaa Xaa Xaa Tyr Xaa Leu Xaa Gly Asn Xaa Xaa Xaa Xaa Xaa Xaa
65                  70                  75                  80
Xaa Xaa Xaa Xaa Xaa Xaa Met Xaa Xaa Xaa Xaa Tyr Xaa Phe Tyr Xaa
                85                  90                  95
Ser Lys Xaa Xaa Glu Phe Xaa Asp Thr Xaa Xaa Met Xaa Leu Xaa Xaa
            100                 105                 110
Xaa Xaa Xaa Gln Xaa Xaa Xaa Leu His Val Tyr His His Xaa Ser Ile
        115                 120                 125
Xaa Xaa Ile Trp Xaa Xaa Ile Xaa Xaa Xaa Ala Pro Gly Gly Xaa Ala
    130                 135                 140
Tyr Phe Ser Xaa Xaa Leu Asn Ser Xaa Xaa His Val Xaa Xaa Tyr Xaa
145                 150                 155                 160
Tyr Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                165                 170                 175
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Thr Xaa Xaa Gln Xaa Xaa Gln
            180                 185                 190
Phe Xaa Xaa Xaa Xaa Xaa Gln Xaa Xaa Tyr Xaa Xaa Xaa Xaa Xaa Xaa
        195                 200                 205
Xaa Tyr Pro Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Tyr Met Xaa Xaa
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    210                 215                 220
xaa Leu xaa Leu Phe Gly Asn Phe xaa xaa xaa xaa xaa xaa xaa xaa
225                 230                 235                 240
xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Lys
                245                 250
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<210> 186
<211> 60
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (3)..(4)
<223> xaa in position 3 to 4 is any amino acid
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Leu, Met or Val
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is any amino acid
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is Phe or Ile
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Ile, Leu or Val
<220>
<221> Variant
<222> (15)..(18)
<223> xaa in position 15 to 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Lys or Arg
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Ile, Leu or Val
<220>
<221> Variant
<222> (22)..(22)
<223> xaa in position 22 is Ser or Thr
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Phe or Val
<220>
<221> Variant
<222> (26)..(26)
<223> xaa in position 26 is Ile or Val
<220>
<221> Variant
<222> (30)..(30)
<223> xaa in position 30 is Ala or Ser
<220>
<221> Variant
<222> (31)..(31)
<223> xaa in position 31 is Ser or Thr
<220>
<221> Variant
<222> (33)..(34)
<223> xaa in position 33 to 34 is any amino acid

<220>
<221> Variant
<222> (37)..(37)
<223> xaa in position 37 is any amino acid
<220>
<221> Variant
<222> (38)..(38)
<223> xaa in position 38 is Ala, Ile or Leu
<220>
<221> Variant
<222> (39)..(39)
<223> xaa in position 39 is Ile or Val
<220>
<221> Variant
<222> (40)..(40)
<223> xaa in position 40 is Ala or Thr
<220>
<221> Variant
<222> (41)..(42)
<223> xaa in position 41 to 42 is any amino acid
<220>
<221> Variant
<222> (45)..(45)
<223> xaa in position 45 is Gly or Asn
<220>
<221> Variant
<222> (47)..(47)
<223> xaa in position 47 is Asp or Glu
<220>
<221> Variant
<222> (50)..(50)
<223> xaa in position 50 is Phe or Trp
<220>
<221> Variant
<222> (52)..(52)
<223> xaa in position 52 is Ala or Thr
<220>
<221> Variant
<222> (53)..(53)
<223> xaa in position 53 is Ala or Ile
<220>
<221> Variant
<222> (57)..(57)
<223> xaa in position 57 is any amino acid
<220>
<221> Variant
<222> (58)..(58)
<223> xaa in position 58 is Ile or Val

<400> 186
Ser Lys xaa xaa Glu Phe xaa Asp Thr xaa xaa Met xaa Leu xaa xaa
  1   5   10   15
xaa xaa xaa Gln xaa xaa xaa Leu His xaa Tyr His His xaa xaa Ile
            20   25   30
xaa xaa Ile Trp xaa xaa xaa xaa xaa xaa Ala Pro xaa Gly xaa Ala
        35   40   45
Tyr xaa Ser xaa xaa Leu Asn Ser xaa xaa His Val
    50   55   60

<210> 187
<211> 23
<212> PRT
<213> Artificial sequence

<220>
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<222> (3)..(3)

<223> xaa in position 3 is Leu or Met
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Phe or Tyr
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Ala or Cys
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is any amino acid
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Glu or Gly
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is any amino acid
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is Ala, Ile or Val
<220>
<221> Variant
<222> (11)..(11)
<223> xaa in position 11 is any amino acid
<220>
<221> Variant
<222> (12)..(12)
<223> xaa in position 12 is Ala or Gln
<220>
<221> Variant
<222> (14)..(17)
<223> xaa in position 14 to 17 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is any amino acid
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Leu or Val
<220>
<221> Variant
<222> (21)..(21)
<223> xaa in position 21 is Phe or Trp

<400> 187
Leu Ser xaa xaa Met xaa xaa xaa xaa xaa xaa xaa Ala xaa xaa xaa
  1   5   10   15
xaa Tyr xaa xaa xaa Gly Asn
            20

<210> 188
<211> 24
<212> PRT
<213> Artificial sequence

<220>
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<222> (3)..(4)
<223> xaa in position 3 to 4 is any amino acid
<220>
<221> Variant

<222> (5)..(5)
<223> xaa in position 5 is Ile or Leu
<220>
<221> Variant
<222> (6)..(7)
<223> xaa in position 6 to 7 is any amino acid
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Ile or Leu
<220>
<221> Variant
<222> (10)..(11)
<223> xaa in position 10 to 11 is any amino acid
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ser or Thr
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Leu or Met
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (21)..(22)
<223> xaa in position 21 to 22 is any amino acid
<220>
<221> Variant
<222> (24)..(24)
<223> xaa in position 24 is Phe or Tyr

<400> 188
Tyr Pro xaa xaa xaa xaa xaa xaa Leu xaa xaa Tyr Met xaa xaa xaa
  1   5   10   15
Leu xaa Leu Phe xaa xaa Phe xaa
            20

<210> 189
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Ile or Leu
<220>
<221> Variant
<222> (4)..(5)
<223> xaa in position 4 to 5 is any amino acid
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Leu or Met
<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is any amino acid
<220>

<221> Variant
<222> (11)..(14)
<223> xaa in position 11 to 14 is any amino acid
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<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Gly, Ile or Val
<220>
<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is Ala or Ser
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is any amino acid
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Trp or Tyr
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Ala or Asp

<400> 189
Tyr xaa Thr xaa xaa Gln xaa xaa Gln Phe xaa xaa xaa xaa xaa Gln
  1   5   10   15
xaa xaa xaa xaa
            20

<210> 190
<211> 18
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(5)
<223> xaa in position 2 to 5 is any amino acid
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is any or no amino acid
<220>
<221> Variant
<222> (8)..(9)
<223> xaa in position 8 to 9 is any amino acid
<220>
<221> Variant
<222> (11)..(12)
<223> xaa in position 11 to 12 is any amino acid
<220>
<221> Variant
<222> (13)..(13)
<223> xaa in position 13 is Phe or Leu
<220>
<221> Variant
<222> (14)..(14)
<223> xaa in position 14 is any amino acid
<220>
<221> Variant
<222> (15)..(15)
<223> xaa in position 15 is Ile or Leu
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is any amino acid

<400> 190
Lys xaa xaa xaa xaa xaa Pro xaa xaa Leu xaa xaa xaa xaa xaa xaa
  1   5   10   15
His Asn

<210> 191
<211> 18
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Pro or Thr
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is any amino acid
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Glu or Pro
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ile, Leu, Met or Val
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Leu, Met or Val
<220>
<221> Variant
<222> (7)..(8)
<223> xaa in position 7 to 8 is any amino acid
<220>
<221> Variant
<222> (9)..(9)
<223> xaa in position 9 is Ile, Leu or Val
<220>
<221> Variant
<222> (10)..(11)
<223> xaa in position 10 to 11 is any amino acid
<220>
<221> Variant
<222> (14)..(15)
<223> xaa in position 14 to 15 is any amino acid
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<221> Variant
<222> (17)..(17)
<223> xaa in position 17 is any amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Gly or Leu

<400> 191
Ser xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Tyr Leu xaa xaa Val
  1   5   10   15
xaa xaa

<210> 192
<211> 10
<212> PRT

<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Ala, Gly or Asn
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is any amino acid
<220>
<221> Variant
<222> (4)..(4)
<223> xaa in position 4 is Ile, Leu or Met
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Ile, Leu or Val
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Trp or Tyr
<220>
<221> Variant
<222> (7)..(7)
<223> xaa in position 7 is Ile, Leu or Val
<220>
<221> Variant
<222> (10)..(10)
<223> xaa in position 10 is Phe, Met or Val

<400> 192
Met Xaa Xaa Xaa Xaa Xaa Xaa Phe Tyr Xaa
1 5 10

<210> 193
<211> 1086
<212> DNA
<213> Phytophthora infestans

<220>
<221> CDS
<222> (1)..(1086)

<400> 193
atg gcg acg aag gag gcg tat gtg ttc ccc act ctg acg gag atc aag 48
Met Ala Thr Lys Glu Ala Tyr Val Phe Pro Thr Leu Thr Glu Ile Lys
1 5 10 15
cgg tcg cta cct aaa gac tgt ttc gag gct tcg gtg cct ctg tcg ctc 96
Arg Ser Leu Pro Lys Asp Cys Phe Glu Ala Ser Val Pro Leu Ser Leu
20 25 30
tac tac acc gtg cgt tgt ctg gtg atc gcg gtg gct cta acc ttc ggt 144
Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ala Leu Thr Phe Gly
35 40 45
ctc aac tac gct cgc gct ctg ccc gag gtc gag agc ttc tgg gct ctg 192
Leu Asn Tyr Ala Arg Ala Leu Pro Glu Val Glu Ser Phe Trp Ala Leu
50 55 60
gac gcc gca ctc tgc acg ggc tac atc ttg ctg cag ggc atc gtg ttc 240
Asp Ala Ala Leu Cys Thr Gly Tyr Ile Leu Leu Gln Gly Ile Val Phe
65 70 75 80
tgg ggc ttc ttc acg gtg ggc cac gat gcc ggc cac ggc gcc ttc tcg 288
Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala Phe Ser
85 90 95
cgc tac cac ctg ctt aac ttc gtg gtg ggc act ttc atg cac tcg ctc 336
Arg Tyr His Leu Leu Asn Phe Val Val Gly Thr Phe Met His Ser Leu
100 105 110
atc ctc acg ccc ttc gag tcg tgg aag ctc acg cac cgt cac cac cac 384
Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His His His

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          115                 120                 125
aag aac acg ggc aac att gac cgt gac gag gtc ttc tac ccg caa cgc        432
Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Val Phe Tyr Pro Gln Arg
      130                 135                 140
aag gcc gac gac cac ccg ctg tct cgc aac ctg att ctg gcg ctc ggg        480
Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala Leu Gly
145                 150                 155                 160
gca gcg tgg ctc gcc tat ttg gtc gag ggc ttc cct cct cgt aag gtc        528
Ala Ala Trp Leu Ala Tyr Leu Val Glu Gly Phe Pro Pro Arg Lys Val
                  165                 170                 175
aac cac ttc aac ccg ttc gag cct ctg ttc gtg cgt cag gtg tca gct        576
Asn His Phe Asn Pro Phe Glu Pro Leu Phe Val Arg Gln Val Ser Ala
              180                 185                 190
gtg gta atc tct ctt ctc gcc cac ttc ttc gtg gcc gga ctc tcc atc        624
Val Val Ile Ser Leu Leu Ala His Phe Phe Val Ala Gly Leu Ser Ile
          195                 200                 205
tat ctg agc ctc cag ctg ggc ctt aag acg atg gca atc tac tac tat        672
Tyr Leu Ser Leu Gln Leu Gly Leu Lys Thr Met Ala Ile Tyr Tyr Tyr
      210                 215                 220
gga cct gtt ttt gtg ttc ggc agc atg ctg gtc att acc acc ttc cta        720
Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu
225                 230                 235                 240
cac cac aat gat gag gag acc cca tgg tac gcc gac tcg gag tgg acg        768
His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu Trp Thr
                  245                 250                 255
tac gtc aag ggc aac ctc tcg tcc gtg gac cga tcg tac ggc gcg ctc        816
Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala Leu
              260                 265                 270
att gac aac ctg agc cac aac atc ggc acg cac cag atc cac cac ctt        864
Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gln Ile His His Leu
          275                 280                 285
ttc cct atc att ccg cac tac aaa ctc aag aaa gcc act gcg gcc ttc        912
Phe Pro Ile Ile Pro His Tyr Lys Leu Lys Lys Ala Thr Ala Ala Phe
      290                 295                 300
cac cag gct ttc cct gag ctc gtg cgc aag agc gac gag cca att atc        960
His Gln Ala Phe Pro Glu Leu Val Arg Lys Ser Asp Glu Pro Ile Ile
305                 310                 315                 320
aag gct ttc ttc cgg gtt gga cgt ctc tac gca aac tac ggc gtt gtg       1008
Lys Ala Phe Phe Arg Val Gly Arg Leu Tyr Ala Asn Tyr Gly Val Val
                  325                 330                 335
gac cag gag gcg aag ctc ttc acg cta aag gaa gcc aag gcg gcg acc       1056
Asp Gln Glu Ala Lys Leu Phe Thr Leu Lys Glu Ala Lys Ala Ala Thr
              340                 345                 350
gag gcg gcg gcc aag acc aag tcc acg taa                              1086
Glu Ala Ala Ala Lys Thr Lys Ser Thr
          355                 360
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<210> 194
<211> 361
<212> PRT
<213> Phytophthora infestans

<400> 194

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Met Ala Thr Lys Glu Ala Tyr Val Phe Pro Thr Leu Thr Glu Ile Lys
1               5                  10                  15
Arg Ser Leu Pro Lys Asp Cys Phe Glu Ala Ser Val Pro Leu Ser Leu
            20                  25                  30
Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ala Leu Thr Phe Gly
        35                  40                  45
Leu Asn Tyr Ala Arg Ala Leu Pro Glu Val Glu Ser Phe Trp Ala Leu
    50                  55                  60
Asp Ala Ala Leu Cys Thr Gly Tyr Ile Leu Leu Gln Gly Ile Val Phe
65                  70                  75                  80
Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala Phe Ser
                85                  90                  95
Arg Tyr His Leu Leu Asn Phe Val Val Gly Thr Phe Met His Ser Leu
            100                 105                 110
Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His His His
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        115                 120                 125
Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Val Phe Tyr Pro Gln Arg
    130                 135                 140
Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala Leu Gly
145                 150                 155                 160
Ala Ala Trp Leu Ala Tyr Leu Val Glu Gly Phe Pro Pro Arg Lys Val
                165                 170                 175
Asn His Phe Asn Pro Phe Glu Pro Leu Phe Val Arg Gln Val Ser Ala
            180                 185                 190
Val Val Ile Ser Leu Leu Ala His Phe Phe Val Ala Gly Leu Ser Ile
        195                 200                 205
Tyr Leu Ser Leu Gln Leu Gly Leu Lys Thr Met Ala Ile Tyr Tyr Tyr
    210                 215                 220
Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu
225                 230                 235                 240
His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu Trp Thr
                245                 250                 255
Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala Leu
            260                 265                 270
Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gln Ile His His Leu
        275                 280                 285
Phe Pro Ile Ile Pro His Tyr Lys Leu Lys Lys Ala Thr Ala Ala Phe
    290                 295                 300
His Gln Ala Phe Pro Glu Leu Val Arg Lys Ser Asp Glu Pro Ile Ile
305                 310                 315                 320
Lys Ala Phe Phe Arg Val Gly Arg Leu Tyr Ala Asn Tyr Gly Val Val
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Glu Ala Ala Ala Lys Thr Lys Ser Thr
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Lys His Ser Ile Pro Asn Ala Cys Phe Glu Ser Asn Leu Gly Leu Ser
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ctc tac tac acg gcc cgc gcg atc ttc aac gcg tcg gcc tcg gcg gcg       144
Leu Tyr Tyr Thr Ala Arg Ala Ile Phe Asn Ala Ser Ala Ser Ala Ala
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Leu Leu Tyr Ala Ala Arg Ser Thr Pro Phe Ile Ala Asp Asn Val Leu
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Leu His Ala Leu Val Cys Ala Thr Tyr Ile Tyr Val Gln Gly Val Ile
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ttc tgg ggc ttc ttc acg gtc ggc cac gac tgc ggc cac tcg gcc ttc       288
Phe Trp Gly Phe Phe Thr Val Gly His Asp Cys Gly His Ser Ala Phe
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tcg cgc tac cac agc gtc aac ttt atc atc ggc tgc atc atg cac tct       336
Ser Arg Tyr His Ser Val Asn Phe Ile Ile Gly Cys Ile Met His Ser
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gcg att ttg acg ccg ttc gag agc tgg cgc gtg acg cac cgc cac cac       384
Ala Ile Leu Thr Pro Phe Glu Ser Trp Arg Val Thr His Arg His His
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cac aag aac acg ggc aac att gat aag gac gag atc ttt tac ccg cac       432
His Lys Asn Thr Gly Asn Ile Asp Lys Asp Glu Ile Phe Tyr Pro His
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ggc ggt gcg tgg ttt gtc tac ttg aag gtc ggg tat gcc ccg cgc acg        528
Gly Gly Ala Trp Phe Val Tyr Leu Lys Val Gly Tyr Ala Pro Arg Thr
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atg agc cac ttt gac ccg tgg gac ccg ctc ctc ctt cgc cgc gcg tcg        576
Met Ser His Phe Asp Pro Trp Asp Pro Leu Leu Leu Arg Arg Ala Ser
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gcc gtc atc gtg tcg ctc ggc gtc tgg gcc gcc ttc ttc gcc gcg tac        624
Ala Val Ile Val Ser Leu Gly Val Trp Ala Ala Phe Phe Ala Ala Tyr
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gcg tac ctc aca tac tcg ctc ggc ttt gcc gtc atg ggc ctc tac tac        672
Ala Tyr Leu Thr Tyr Ser Leu Gly Phe Ala Val Met Gly Leu Tyr Tyr
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tat gcg ccg ctc ttt gtc ttt gct tcg ttc ctc gtc att acg acc ttc        720
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ttg cac cac aac gac gaa gcg acg ccg tgg tac ggc gac tcg gag tgg        768
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acg tac gtc aag ggc aac ctc tcg agc gtc gac cgc tcg tac ggc gcg        816
Thr Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala
            260                 265                 270
ttc gtg gac aac ctg agc cac cac att ggc acg cac cag gtc cac cac        864
Phe Val Asp Asn Leu Ser His His Ile Gly Thr His Gln Val His His
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305                 310                 315                 320
atc acg gcc ttc ttc aag acc gcg cac ctc ttt gtc aac tac ggc gct       1008
Ile Thr Ala Phe Phe Lys Thr Ala His Leu Phe Val Asn Tyr Gly Ala
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gtg ccc gag acg gcg cag atc ttc acg ctc aaa gag tcg gcc gcg gcc       1056
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Ala Val Ile Val Ser Leu Gly Val Trp Ala Ala Phe Phe Ala Ala Tyr
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Ala Tyr Leu Thr Tyr Ser Leu Gly Phe Ala Val Met Gly Leu Tyr Tyr
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Tyr Ala Pro Leu Phe Val Phe Ala Ser Phe Leu Val Ile Thr Thr Phe
225                 230                 235                 240
Leu His His Asn Asp Glu Ala Thr Pro Trp Tyr Gly Asp Ser Glu Trp
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Thr Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala
            260                 265                 270
Phe Val Asp Asn Leu Ser His His Ile Gly Thr His Gln Val His His
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Leu Phe Pro Ile Ile Pro His Tyr Lys Leu Asn Glu Ala Thr Lys His
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Phe Ala Ala Ala Tyr Pro His Leu Val Arg Arg Asn Asp Glu Pro Ile
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Ile Thr Ala Phe Phe Lys Thr Ala His Leu Phe Val Asn Tyr Gly Ala
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            20                  25                  30
xaa Arg xaa xaa xaa xaa Ala xaa Ala xaa xaa xaa xaa Leu xaa xaa
        35                  40                  45
Ala Arg xaa xaa Pro xaa xaa xaa xaa xaa xaa xaa Leu xaa Ala xaa
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 65                  70                  75                  80
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                85                  90                  95
xaa xaa Asn Phe xaa xaa Gly xaa xaa Met His Ser xaa Ile Leu Thr
            100                 105                 110
Pro Phe Glu Ser Trp xaa xaa Thr His Arg His His His Lys Asn Thr
        115                 120                 125
Gly Asn Ile Asp xaa Asp Glu xaa Phe Tyr Pro xaa Arg xaa xaa xaa
    130                 135                 140
Asp xaa xaa xaa xaa Arg xaa xaa xaa xaa xaa Leu Gly xaa Ala Trp
145                 150                 155                 160
xaa xaa Tyr Leu xaa xaa Gly xaa xaa Pro Arg xaa xaa xaa His Phe
                165                 170                 175
xaa Pro xaa xaa Pro Leu xaa xaa Arg xaa xaa Ser Ala Val xaa xaa
            180                 185                 190
Ser Leu xaa xaa xaa Ala xaa Phe Phe xaa Ala xaa xaa xaa xaa Tyr
        195                 200                 205
Leu xaa xaa xaa Leu Gly xaa xaa xaa Met xaa xaa Tyr Tyr Tyr xaa
    210                 215                 220
Pro xaa Phe Val Phe xaa Ser xaa Leu Val Ile Thr Thr Phe Leu His
225                 230                 235                 240
His Asn Asp Glu xaa Thr Pro Trp Tyr xaa Asp Ser Glu Trp Thr Tyr
                245                 250                 255
Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala xaa xaa
            260                 265                 270
Asp Asn Leu Ser His xaa Ile Gly Thr His Gln xaa His His Leu Phe
        275                 280                 285
Pro Ile Ile Pro His Tyr Lys Leu xaa xaa Ala Thr xaa xaa Phe xaa
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xaa Ala xaa Pro xaa Leu Val Arg xaa xaa Asp Glu Pro Ile Ile xaa
305                 310                 315                 320
Ala Phe Phe xaa xaa xaa xaa Leu xaa xaa Asn Tyr Gly xaa Val xaa
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35 40 45
xaa His His Leu Phe Pro Ile Ile Pro His Tyr Lys
50 55 60

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Phe Glu Ser Trp xaa xaa Thr His Arg His His His Lys Asn Thr Gly
        35                  40                  45
Asn Ile Asp xaa Asp Glu xaa Phe Tyr Pro xaa Arg
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CONFIRMATION NO. 4050

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Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

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OC00000088054967

Title:METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

Publication No.US-2016-0369290-A1
Publication Date:12/22/2016

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 4

Complete if Known	
Application Number	15/256,914-Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA*	US-60/613,861	N/A	Singh et al.	
	AB*	US-2008/0076164-A1	03-27-2008	Cirpus et al.	
	AC*	US-2013/0116421-A1	05-09-2013	Cirpus et al.	

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	BA	AU-2001239244-B2	08-20-2001	BASF Aktiengesellschaft		See Abstract
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	BC	AU-2003258496-A1	01-23-2004	BASF Plant Science GmbH		See WO2004/005442
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	BE	AU-2004225838-B2	10-14-2004	University of Bristol		See Abstract
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	BH	WO-00/34439-A1	06-15-2000	Washington State University Research Foundation		
	BI	WO-01/85968-A2	11-15-2001	Bioriginal Food & Science Corp.		
	BJ	WO-02/090493-A2	11-14-2002	Abbott Laboratories		
	BK	WO-02/26946-A2	04-04-2002	Bioriginal Food & Science Corp.		
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	BM	WO-2004/005442-A1	01-15-2004	BASF Plant Science GmbH		See Abstract
	BN	WO-2004/057001-A2	07-08-2004	University of Bristol		
	BO	WO-2005/083053-A2	09-09-2005	BASF Plant Science GmbH		See US2013/0116421
	BP	WO-2005/103253-A1	11-03-2005	Commonwealth Scientific and Industrial Research Organisation		
	BQ	WO-2006/008099-A2	01-26-2006	BASF Plant Science GmbH		See US2008/0076164
	BR	WO-2010/057246-A1	05-27-2010	Commonwealth Scientific and Industrial Research Organisation		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 4

Complete if Known	
Application Number	15/256,914-Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	ROBERT, S. S., et al., "Metabolic Engineering of *Arabidopsis* to Produce Nutritionally Important DHA in Seed Oil", Functional Plant Biology, 2005, Vol. 32, pp. 473-479.	
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	CC	WOLFF, R. L., et al., "Arachidonic, Eicosapentaenoic, and Biosynthetically Related Fatty Acids in the Seed Lipids from a Primitive Gymnosperm, *Agathis robusta*", Lipids, 1999, Vol. 34, No. 10, pp. 1083-1097.	
	CD	HONG, H., et al., "High-Level Production of γ-Linolenic Acid in *Brassica juncea* Using a Δ6 Desaturase from *Pythium irregulare*", Plant Physiology, 2002, Vol. 129, pp. 354-362.	
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Complete if Known	
Application Number	15/256,914-Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CN	BEAUDOIN, F., et al., "Production of C_{20} Polyunsaturated Fatty Acids (PUFAs) by Pathway Engineering: Identification of a PUFA Elongase Component from *Caenorhabditis elegans*", Biochemical Society Transactions, 2000, Vol. 28, pp. 661-663.	
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	CP	THURMOND, T. Das J. M., et al., "Polyunsaturated Fatty Acid-Specific Elongation Enzymes", Biochemical Society Transactions, 2000, Vol. 28, pp. 658-660.	
	CQ	SATO, S., et al., "Production of γ-Linolenic Acid and Stearidonic Acid in Seeds of Marker-Free Transgenic Soybean", Crop Science, 2004, Vol. 44, pp. 646-652.	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Complete if Known	
Application Number	15/256,914-Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA1	AGABA, M., et al., "Zebrafish cDNA Encoding Multifunctional Fatty Acid Elongase Involved in Production of Eicosapentaenoic (20:5n-3) and Docosahexaenoic (22:6n-3) Acids", Marine Biotechnology, 2004, Vol. 6, pp. 251-261.	
	CB1	ARMBRUST, E. V., et al., "The Genome of the Diatom *Thalassiosira pseudonana*: Ecology, Evolution, and Metabolism", Science, 2004, Vol. 306, pp. 79-86.	
	CC1	CLOUGH, S. J., et al., "Floral Dip: A Simplified Method for Agrobacterium-Mediated Transformation of *Arabidopsis thaliana*", The Plant Journal, 1998, Vol. 16, No. 6, pp. 735-743.	
	CD1	QIU, X., et al., "Identification of a Δ4 Fatty Acid Desaturase from *Thraustochytrium* sp. Involved in the Biosynthesis of Docosahexanoic Acid by Heterologous Expression in *Saccharomyces cerevisiae* and *Brassica juncea*", The Journal of Biological Chemistry, 2001, Vol. 276, No. 34, pp. 31561-31566.	
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	AF1	DOMERGUE, F., et al., "In Vivo Characterization of the First Acyl-CoA Δ^6-Desaturase from a Member of the Plant Kingdom, the Microalga *Ostreococcus tauri*", Biochem. J., 2005, Vol. 389, pp. 483-490.	
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EFS ID:	28006996
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Hui-Ju Wu/Jamie Jensen-Smith
Filer Authorized By:	Hui-Ju Wu
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	09-JAN-2017
Filing Date:	06-SEP-2016
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Application Type:	Utility under 35 USC 111(a)

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56	Non Patent Literature	Ursin.pdf	62336 9c79a3afbf2c8a3055ee52f917f22d6fa4da23be	no	2
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Information:					
			Total Files Size (in bytes):	134544818	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 074017-0013-01-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: Hope A. Robinson

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 CFR 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits (37 C.F.R. § 1.97(b)(3)).

In accordance with 37 CFR 1.98(a)(2)(ii), Applicant has not submitted copies of U.S. patent applications cited in the attached PTO/SB/08. Applicant submits herewith copies of foreign patents and non-patent literature in accordance with 37 CFR 1.98(a)(2).

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this Information Disclosure statement shall not be construed to be an admission that any patent,

publication or other information referred to therein is "prior art" for this invention unless specifically designated as such. Moreover, Applicant understands that the Examiner will make an independent evaluation of the cited documents.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the listed references.

Applicant believes no fee is due with this submission. However, if a fee is due, the Director is hereby authorized to charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US, from which the undersigned is authorized to draw.

Respectfully submitted,

By___/ *Hui-Ju Wu* /_____

Hui-Ju Wu, Ph.D.
Registration No.: 57,209
Drinker Biddle & Reath LLP
222 Delaware Ave., Ste. 1410
Wilmington, Delaware 19801-1621
(302) 467-4260
(302) 351-6938 (Fax)
Attorney for Applicant

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 01/27/2017

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
01/27/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

IPDocketWM@dbr.com
penelope.mongelluzzo@dbr.com
DBRIPDocket@dbr.com

Office Action Summary	Application No. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *1/9/17*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-22* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-22* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

Restriction/Election

1. The present application is being examined under the pre-AIA first to invent provisions.

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-16 are drawn to oils, lipids and fatty acids, classified in (A61K 31/19).

II. Claims 23-28 are drawn to a method for making oils, lipids and fatty acids, classified in (A61K 31/20).

3. The inventions are distinct, each from the other because of the following reasons: Inventions I and II are related as product and process of making. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for making the product as claimed can be practiced with another materially different product or (2) the product as claimed can be made in a materially different process of making that product (MPEP § 806.05(h)). In the instant case the product can be made in a materially different process such as a bioassay.

4. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

Furthermore, the inventions have acquired a separate status in the art as a separate subject for inventive effect and require independent searches. The search for each of the above inventions is not co-extensive particularly with regard to the literature search. A reference, which would anticipate the invention of one group, would not necessarily anticipate or make obvious the other group. Moreover, as to the question of burden of search, classification of subject matter is merely one indication of the burdensome nature of the search involved. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification and because of their recognized divergent subject matter, election of a single group for examination purposes as indicated is proper.

5. The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim will be rejoined in accordance with the provisions of MPEP § 821.04. **Process claims that depend from or otherwise include all the limitations of the patentable product** will be entered as a matter of right if the amendment is presented prior to final rejection or allowance, whichever is earlier. Amendments submitted after final

rejection are governed by 37 CFR 1.116; amendments submitted after allowance are governed by 37 CFR 1.312.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103, and 112. Until an elected product claim is found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowed product claim will not be rejoined. See "Guidance on Treatment of Product and Process Claims in light of *In re Ochiai, In re Brouwer* and 35 U.S.C. § 103(b)," 1184 O.G. 86 (March 26, 1996). Additionally, in order to retain the right to rejoinder in accordance with the above policy, Applicant is advised that the process claims should be amended during prosecution either to maintain dependency on the product claims or to otherwise include the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Species election:

6. This application contains claims directed to the following patentably distinct species of components: oils, lipids and fatty acids with the accompanying DNAs. For

examination on the merits an election is required of a single specific component for each category in the aforementioned claims (specific oil, lipid and fatty acid). The species are independent or distinct because they represent different components with different structures and function. In addition, these species are not obvious variants of each other based on the current record.

Claim(s) 1 is/are generic to the following disclosed patentably distinct species: of oil, lipid and fatty acid. The species are independent or distinct because as disclosed the different species have mutually exclusive characteristics for each identified species (structure and function are distinct). In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply: the search is not coextensive.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or a grouping of patentably indistinct species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species** or grouping of patentably indistinct species, including any claims subsequently added. An argument

that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(l).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday from 9:00 a.m. to 5:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi, can be reached at (571) 272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Hope A. Robinson/

Primary Examiner, Art Unit 1652

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 02/07/2017
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
02/07/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

IPDocketWM@dbr.com
penelope.mongelluzzo@dbr.com
DBRIPDocket@dbr.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	15/256,914	CIRPUS ET AL.	
	Examiner	Art Unit	
	HOPE ROBINSON	1652	

All participants (applicant, applicant's representative, PTO personnel):

(1) *HOPE ROBINSON*. (3)_____.

(2) *Hui-Ju Wu*. (4)_____.

Date of Interview: *31 January 2017*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1-22*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Upon due reconsideration it was noted that Group II has an inadvertent typographical error and should represent claims 17-22 and not claims 23-28. In addition the species portion of the restriction was discussed and clarified. Ms. Wu plans to file an appropriate amendment to elect an invention as well as an oil, lipid or fatty acid with a specific composition.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/HOPE ROBINSON/ Primary Examiner, Art Unit 1652	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Docket No.: 074017-0013-01-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: Hope A. Robinson

AMENDMENT AND RESPONSE TO RESTRICTION REQUIREMENT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Communication dated January 27, 2017, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

Listing of Claims:

1. (Original) Oils, lipids and/or fatty acids produced by a transgenic Brassica plant, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant, wherein said polyunsaturated fatty acids comprise at least 20% by weight of eicosapentaenoic acid (EPA), at least 2% by weight of docosapentaenoic acid (DPA), and/or at least 4% by weight of docosahexaenoic acid (DHA) based on the total fatty acids in the transgenic plant in form of triacylglycerides.

2. (Original) The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-1, sn-2 or sn-3 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 1.5% by weight of DPA is present in the sn-1, sn-2 or sn-3 position; and/or

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-1, sn-2 or sn-3 position.

3. (Original) The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-2 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 3% by weight of DPA is present in the sn-2 position; and/or

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-2 position.

4. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise:

a) at least 20% by weight of EPA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides;

b) at least 20% by weight of EPA and at least 4% by weight of DHA based on the

total fatty acids in the transgenic plant in form of triacylglycerides; or

c) at least 2% by weight of DPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

5. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

6. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

7. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

8. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

9. (Original) Oils, lipids and/or fatty acids produced by a transgenic Brassica plant, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

10. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant.

11. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 20% by weight of EPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

12. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

13. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

14. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

15. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

16. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

17. (Original) A method for producing oils, lipids and/or fatty acids of claim 1, comprising expressing in a Brassica plant a nucleic acid encoding a Δ6-desaturase, a nucleic acid encoding a Δ5-desaturase, a nucleic acid encoding a Δ6-elongase, a nucleic acid encoding a ω3-desaturase, a nucleic acid encoding a Δ5-elongase, and a nucleic acid encoding a Δ4-desaturase, wherein said nucleic acid encoding a Δ5-elongase is codon-optimized by adapting to the codon usage of Brassica.

18. (Original) The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a nucleotide sequence having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 64, and wherein said nucleotide sequence is obtained by adapting at least 30% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of Brassica.

19. (Original) The method of claim 18, wherein said nucleotide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 64, or wherein said nucleotide sequence encodes a polypeptide having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65.

20. (Original) The method of claim 18, wherein said nucleotide sequence is adapted taking

into account the natural frequency of individual codons.

21. (Original) The method of claim 18, wherein said nucleotide sequence is obtained by adapting at least 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of Brassica.

22. (Original) The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a translated section coding for a protein having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65, and wherein the translated section comprises a nucleotide sequence obtained by adapting at least 30% or 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of Brassica.

23. (New) The oils, lipids and/or fatty acids of claim 1, comprising:

a) at least 4% by weight of DHA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant;

b) at least 4% by weight of DHA and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant; or

c) at least 4% by weight of DHA, at least 2% by weight of DPA, and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant.

REMARKS

After entry of this amendment, claims 1-23 are pending. New claim 23 has been added and finds support *inter alia* in the original claims and the as-filed specification. No new matter has been added.

In response to the restriction requirement set forth in the Office Communication dated January 27, 2017, Applicant hereby elects Group I with claims drawn to oils, lipids and fatty acids for further prosecution without traverse. Applicant believes that claims 1-16 and new claim 23 are encompassed by the elected Group. Applicant further elects oils and at least 4% by weight of docosahexaenoic acid (DHA) as species for further prosecution without traverse. Applicant believes that at least claims 1-5, 7-10, 13, 14, 16 and 23 read on the elected species. As noted by the Examiner at page 6 of the Office Action, upon allowance of a generic claim, claims to additional species which are written in dependent form or otherwise require all the limitations of an allowed generic claim would be considered.

Applicant reserves all rights to pursue the non-elected and/or cancelled subject matter in one or more continuing applications.

This response is filed within the two-month response period from the mailing of the Office Communication. No fee is believed due. However, if a fee is due, please charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US, from which the undersigned is authorized to draw.

Respectfully submitted,

By___/ *Hui-Ju Wu* /_____

Hui-Ju Wu, Ph.D.
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Attorney for Applicant

#87,537,224

Electronic Acknowledgement Receipt

EFS ID:	28714125
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Hui-Ju Wu/Jamie Jensen-Smith
Filer Authorized By:	Hui-Ju Wu
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	23-MAR-2017
Filing Date:	06-SEP-2016
Time Stamp:	10:48:38
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		ResponseToRestrictionRequirement.pdf	36455 e15a8e977b74f9357f946e92f03295a57f08d2dc	yes	6

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Election / Restriction Filed	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	6
Warnings:			
Information:			
	Total Files Size (in bytes):	36455	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/256,914	Filing Date 09/06/2016	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT

03/23/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 23	Minus	** 22	= 1	X $80 =	80
Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	80

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

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Document code: WFEE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 07/21/2017

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
07/21/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

IPDocketWM@dbr.com
penelope.mongelluzzo@dbr.com
DBRIPDocket@dbr.com

Office Action Summary	Application No. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *3/23/17*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-23* is/are pending in the application.

5a) Of the above claim(s) *6,11,12,15 and 17-22* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-5,7-10,13,14,16 and 23* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☒ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☒ All b)☐ Some** c)☐ None of the:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Application Status

1. The present application is being examined under the pre-AIA first to invent provisions.

2. Applicant's election without traverse of Group I (claims 1-5, 7-10, 13-14, 16 and 23 with species DHA), is acknowledged.

Claim Disposition

3. Claim 23 has been added. Claims 1-23 are pending. Claims 1-5, 7-10, 13-14, 16 and 23 are under examination. Claims 6, 11-12, 15 and 17-22 are withdrawn from further consideration pursuant to 37 CFR 1.12(b), as being drawn to a non-elected invention, there being no allowable generic or linking claim.

4. The Amendment filed on September 6, 2016 has been received and entered.

Specification

5. The specification is objected to because of the following informalities:

The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The following is suggested: "Oils, lipids and fatty acids produced in transgenic *Brassica* plant".

The specification is objected to because the organism names are not italicized, see for example pages 23-28, page 74 and throughout the specification; and also need to have the spelled out meaning at the first occurrence (see i.e. E. coli on page 74, for example).

The specification is objected because the spelled out meaning is not provided in the following paragraph for the acronym (emphasis added):

“Paragraph [0396] “It was possible to find **VLCPUFA** in these lipids too, with an accumulation of EPA in the sn-2 position being observed. DHA was to be found only in the digalactodiacylglycerols (DGDG) and was undetectable in the monogalactodiacylglycerols (MGDG) (Table 5). The distribution of **VLCPUFA** in galactolipids, a compartment in which these fatty acids were not expected, shows the dynamics of the synthesis and the later transformation. **VLCPUFA** in polar lipids are of particular nutritional value because they can be absorbed better in the intestines of

mammals than the neutral lipids", see page 79 of the specification.

Correction is required.

Information Disclosure Statement

6. The Information Disclosure Statements filed on January 9, 2017 and September 6, 2016, have been received and entered. The references cited on the PTO-1449 Form have been considered by the examiner and a copy is attached to the instant Office action. Note that a reference has been lined through on the form as it represents an improper citation, wherein digits are missing from the document number. Applicant is urged to resubmit the 1449 for consideration.

Claim Objection

7. Claims 1 and 9 are objected to because of the following informalities:

For clarity it is suggested that claims 1 and 9 are amended to italicize the names of the organism.

Correction is required.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

8. The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) and the Intellectual Property and High Technology Technical Amendments Act of 2002 do not apply when the reference is a U.S. patent resulting directly or indirectly from an international application filed before November 29, 2000. Therefore, the prior art date of the reference is determined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (**pre-AIPA** 35 U.S.C. 102(e)).

Claim(s) 1-5, 7-10, 13-14, 16 and 23 is/are rejected under pre-AIA 35 U.S.C. 102(e) as being anticipated by Cirpus et al. (US Patent No. 9,458,436, 2004).

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under pre-AIA 35 U.S.C. 102(e). This rejection under pre-AIA 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor or joint inventors (i.e., the

inventive entity) of this application and is thus not the invention “by another,” or if the same invention is not being claimed, by an appropriate showing under 37 CFR 1.131(a).

The present invention relates to oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant and comprise certain weight percent of EPA, DHA and DPA (see instant claim 1 for example). The patented invention is directed to a process for the production of polyunsaturated fatty acids in the seed of transgenic plants by introducing, into the organism, nucleic acids which encode polypeptides with a .omega.3-desaturase, .DELTA.12-desaturase, .DELTA.6-desaturase, .DELTA.6-elongase, .DELTA.5-desaturase, .DELTA.5-elongase and/or .DELTA.4-desaturase activity. The invention furthermore relates to recombinant nucleic acid molecules comprising the nucleic acid sequences which encode the aforementioned polypeptides, either jointly or individually, and transgenic plants which comprise the aforementioned recombinant nucleic acid molecules. Furthermore, the invention relates to the generation of a transgenic plant and to oils, lipids and/or fatty acids with an elevated content of polyunsaturated fatty acids, in particular arachidonic acid, eicosapentaenoic acid and/or docosahexaenoic acid, as the result of the expression of the elongases and desaturases used in the process according to the invention (see abstract and paragraph 6 of the patent).

In addition, the patent discloses a percentage for DHA that corresponds to the claimed invention. At paragraph 89 of the patent it is disclosed that, “DHA is produced in the process according to the invention in a content of at least 0.01 or 0.02% by weight, advantageously at least 0.03 or 0.05% by weight, advantageously at least 0.09 or 0.1%

by weight especially preferably at least 0.2 or 0.3% by weight and most preferably at least 0.35% by weight based on the total lipid content in the seeds of the transgenic plants. At paragraph 8 of the patent it is disclosed that there is a higher content of LCPUFAs. DHA in the form of triacylglycerides is disclosed (see paragraph 17, for example). The weight percent recited in for example claim 9 is obvious based on the disclosure in paragraphs 59-80. Therefore, the limitations of the claims are met by the reference.

Conclusion

9. No claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday from 9:00 a.m. to 5:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi, can be reached at (571) 272-0956.

The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Hope A. Robinson/

Primary Examiner, Art Unit 1652

Notice of References Cited	Application/Control No. 15/256,914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-9,458,436 B2	10-2016	Cirpus; Petra	A23D9/00	1/1
	B	US-				
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /H.A.R./

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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	4

Complete if Known	
Application Number	15/256,914-Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA*	US-60/613,861	N/A	Singh et al.	
	AB*	US-2008/0076164-A1	03-27-2008	Cirpus et al.	
	AC*	US-2013/0116421-A1	05-09-2013	Cirpus et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA	AU-2001239244-B2	08-20-2001	BASF Aktiengesellschaft		See Abstract
	BB	AU-2003232512-B2	11-17-2003	BASF Plant Science GmbH		See Abstract
	BC	AU-2003258496-A1	01-23-2004	BASF Plant Science GmbH		See WO2004/005442
	BD	AU-2004215705-B2	09-10-2004	BASF Plant Science GmbH		See Abstract
	BE	AU-2004225838-B2	10-14-2004	University of Bristol		See Abstract
	BF	AU-2004227075-B8	10-21-2004	BASF Plant Science GmbH		See Abstract
	BG	AU-2005217080-B2	09-09-2005	BASF Plant Science GmbH		See Abstract
	BH	WO-00/34439-A1	06-15-2000	Washington State University Research Foundation		
	BI	WO-01/85968-A2	11-15-2001	Bioriginal Food & Science Corp.		
	BJ	WO-02/090493-A2	11-14-2002	Abbott Laboratories		
	BK	WO-02/26946-A2	04-04-2002	Bioriginal Food & Science Corp.		
	BL	WO-03/102138-A2	12-11-2003	Abbott Laboratories		
	BM	WO-2004/005442-A1	01-15-2004	BASF Plant Science GmbH		See Abstract
	BN	WO-2004/057001-A2	07-08-2004	University of Bristol		
	BO	WO-2005/083053-A2	09-09-2005	BASF Plant Science GmbH		See US2013/0116421
	BP	WO-2005/103253-A1	11-03-2005	Commonwealth Scientific and Industrial Research Organisation		
	BQ	WO-2006/008099-A2	01-26-2006	BASF Plant Science GmbH		See US2008/0076164
	BR	WO-2010/057246-A1	05-27-2010	Commonwealth Scientific and Industrial Research Organisation		

Examiner Signature	/HOPE A ROBINSON/	Date Considered	06/24/2017

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	2	of	4

Complete if Known	
Application Number	15/256,914-Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	ROBERT, S. S., et al., "Metabolic Engineering of *Arabidopsis* to Produce Nutritionally Important DHA in Seed Oil", Functional Plant Biology, 2005, Vol. 32, pp. 473-479.	
	CB	STIRN, S., et al., "Genetically Modified Plants", Chapter 2 in "Genetically Engineered Food: Methods and Detection", HELLER, K. J., Ed., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, Published Online January 7, 2005, pp. 26-61.	
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	CF	SAYANOVA, O. V., et al., "Eicosapentaenoic Acid: Biosynthetic Routes and the Potential for Synthesis in Transgenic Plants", Phytochemistry, 2004, Vol. 65, pp. 147-158.	
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	CN	BEAUDOIN, F., et al., "Production of C_{20} Polyunsaturated Fatty Acids (PUFAs) by Pathway Engineering: Identification of a PUFA Elongase Component from *Caenorhabditis elegans*", Biochemical Society Transactions, 2000, Vol. 28, pp. 661-663.	
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	CP	THURMOND, T. Das J. M., et al., "Polyunsaturated Fatty Acid-Specific Elongation Enzymes", Biochemical Society Transactions, 2000, Vol. 28, pp. 658-660.	
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	CS	WALLIS, J. G., et al., "The Δ^8-Desaturase of *Euglena gracilis*: An Alternate Pathway for Synthesis of 20-Carbon Polyunsaturated Fatty Acids", Archives of Biochemistry and Biophysics, 1999, Vol. 365, No. 2, pp. 307-316.	
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	CU	QI, B., et al., "Production of Very Long Chain Polyunsaturated Omega-3 and Omega-6 Fatty Acids in Plants", Nature Biotechnology, 2004, Vol. 22, No. 6, pp. 739-745.	
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	CW	LEONARD, A. E., et al., "cDNA Cloning and Characterization of Human Δ^5-Desaturase Involved in the Biosynthesis of Arachidonic Acid", Biochem. J., 2000, Vol. 347, pp. 719-724.	
	CX	HONG, H., et al., "Isolation and Characterization of a Δ5 FA Desaturase from *Pythium irregulare* by Heterologous Expression in *Saccharomyces cerevisiae* and Oilseed Crops", Lipids, 2002, Vol. 37, No. 9, pp. 863-868.	
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	CZ	LEONARD, A. E., et al., "Identification and Expression of Mammalian Long-Chain PUFA Elongation Enzymes", Lipids, 2002, Vol. 37, No. 8, pp. 733-740.	

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	CA1	AGABA, M., et al., "Zebrafish cDNA Encoding Multifunctional Fatty Acid Elongase Involved in Production of Eicosapentaenoic (20:5n-3) and Docosahexaenoic (22:6n-3) Acids", Marine Biotechnology, 2004, Vol. 6, pp. 251-261.	
	CB1	ARMBRUST, E. V., et al., "The Genome of the Diatom *Thalassiosira pseudonana*: Ecology, Evolution, and Metabolism", Science, 2004, Vol. 306, pp. 79-86.	
	CC1	CLOUGH, S. J., et al., "Floral Dip: A Simplified Method for Agrobacterium-Mediated Transformation of *Arabidopsis thaliana*", The Plant Journal, 1998, Vol. 16, No. 6, pp. 735-743.	
	CD1	QIU, X., et al., "Identification of a Δ4 Fatty Acid Desaturase from *Thraustochytrium* sp. Involved in the Biosynthesis of Docosahexanoic Acid by Heterologous Expression in *Saccharomyces cerevisiae* and *Brassica juncea*", The Journal of Biological Chemistry, 2001, Vol. 276, No. 34, pp. 31561-31566.	
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	AG1	VENEGAS-CALERÓN, M., et al., "An Alternative to Fish Oils: Metabolic Engineering of Oil-Seed Crops to Produce Omega-3 Long Chain Polyunsaturated Fatty Acids", Progress in Lipid Research, 2010, Vol. 49, pp. 108-119.	
	AH1	MEYER, A., et al., "Biosynthesis of Docosahexaenoic Acid in *Euglena gracilis*: Biochemical and Molecular Evidence for the Involvement of a Δ4-Fatty Acyl Group Desaturase", Biochemistry, 2003, Vol. 42, pp. 9779-9788.	
	AI1	DOMERGUE, F., et al., "Acyl Carriers Used as Substrates by the Desaturases and Elongases Involved in Very Long-chain Polyunsaturated Fatty Acids Biosynthesis Reconstituted in Yeast", The Journal of Biological Chemistry, 2003, Vol. 278, No. 37, pp. 35115-35126.	
	AJ1	GUNSTONE, F. D., "Vegetable Oils", In: Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Set, pp. 213-267, Ed. Shahidi, John Wiley & Sons, Inc., 2005.	
	AK1	URSIN, V., et al., "Production of Beneficial Dietary Omega-3 and Omega 6 Fatty Acids in Transgenic Canola", Abstract No. 49, 14th International Symposium Plant Lipids, 2000.	

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Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA*	US-5,614,393	03-25-1997	Thomas T. L. et al.	
	AB*	US-6,043,411	03-28-2000	Nishizawa et al.	
	AC*	US-2004/0111763	06-10-2004	Heinz et al.	
	AD*	US-6,884,921-B2	04-26-2005	Browse et al.	
	AE*	US-6,459,018-B1	10-01-2002	Knutzon	
	AF*	US-7,550,286-B2	06-23-2009	Damude et al.	
	AG*	US-7,777,098-B2	08-17-2010	Cirpus et al.	
	AH*	US-2004/0172682-A1	09-02-2004	Kinney et al.	
	AI*	US-2004/0049805-A1	03-11-2004	Lerchl et al.	
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	AK*	US-2008/0155705-A1	06-26-2008	Zank et al.	
	AL*	US-2009/0222951-A1	09-03-2009	Cirpus et al.	
	AM*	US-2010/0021976-A1	01-28-2010	Lerchl et al.	

FOREIGN PATENT DOCUMENTS

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	BA**	WO-91/13972	09-19-1991	Calgene Inc.		
	BB**	WO-93/06712	04-15-1993	Rhone-Poulenc Agrochimie		
	BC**	WO-93/11245	06-10-1993	E.I. DuPont De Nemours and Co.		
	BD**	EP-0 550 162	07-07-1993	Pioneer Hi-Bred International, Inc.		
	BE**	WO-94/11516	05-26-1994	E.I. duPont de Nemours And Company		
	BF**	WO-94/18337	08-18-1994	Monsanto Company & Michigan State University		
	BG**	WO-96/21022	07-11-1996	Rhone-Poulenc Agrochimie		
	BH**	WO-97/21340	06-19-1997	Cargill, Inc.		
	BI**	WO-97/30582	08-28-1997	Carnegie Institution Of Washington & Monsanto Co., Inc.		
	BJ**	WO-01/85968-A2	11-15-2001	Bioriginal Food & Science Corp.		
	BK**	EP-0 794 250	09-10-1997	Soremartec S.A. & Ferrero S.p.A.		

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Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	BL**	WO-98/46776	10-22-1998	Calgene LLC		
	BM**	WO-98/46764	10-22-1998	Calgene LLC & Abbott Laboratories		
	BN**	WO-98/46763	10-22-1998	Calgene LLC & Abbott Laboratories		
	BO**	WO-98/46765	10-22-1998	Calgene LLC & Abbott Laboratories		
	BP**	WO-99/27111	06-03-1999	University of Bristol		
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	BZ**	WO-2004/071467	08-26-2004	E. I. duPont de Nemours and Company		
	BA1**	WO-02/057464-A2	07-25-2002	BASF Plant Science GmbH		See US2004/0049805
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	BH1**	WO-2005/012316-A2	02-10-2005	BASF Plant Science GmbH		See Abstract
	BI1**	CA-2559360-A1	09-09-2005	BASF Plant Science GmbH		
	BJ1**	WO-2005/103253-A1	11-03-2005	Commonwealth Scientific And Industrial Research Organisation		

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	CA**	"Danio rerio Polyunsaturated Fatty Acid Elongase Protein", GenBank Database Accession No. AAN77156, February 15, 2006.	
	CB**	Cronan, J.E. et al., "Biosynthesis of Membrane Lipids", in "E. coli und Salmonella", Section B2, Neidhardt, F.C. et al. eds., ASM Press, Washington, DC, (1996), pp. 612-636.	
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	CD**	Wada, H. et al., "Enhancement of Chilling Tolerance of a Cyanobacterium by Genetic Manipulation of Fatty Acid Desaturation", Nature 347 (1990), pp. 200-203.	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 4 of 6

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	CS**	Sakuradani, E. et al., "Δ6-Fatty Acid Desaturase from an Arachidonic Acid-Producing *Mortierella* Fungus Gene Cloning and Its Heterologous Expression in a Fungus, *Aspergillus*", Gene 238 (1999), pp. 445-453.	
	CT**	Kinney, A.J., "Genetic Engeering of Oilseeds for Desired Traits", in "Genetic Engineering, Principles and Methods", Vol. 19, Editor: J. Setlow, (1997), pp. 149-166.	
	CU**	Voelker, T., "Plant Acyl-ACP Thioesterases: Chain-Length Determining Enzymes in Plant Fatty Acid Biosynthesis", in "Genetic Engineering, Principles and Methods", Vol. 18, Editor: J. Setlow, (1996), pp. 111-113.	
	CV**	Stukey, J.E. et al., "The *OLE1* Gene of *Saccharomyces cerevisiae* Encodes the Δ9 Fatty Acid Desaturase and Can Be Functionally Replaced by the Rat Stearoyl-CoA Desaturase Gene", The Jounal of Biological Chemistry 265:33 (1990), pp. 20144-20149.	
	CW**	Zank, T.K. et al., "Cloning and Functional Expression of the First Plant Fatty Acid Elongase Specific for Δ^6-Polyunsaturated Fatty Acids", Biochemical Society Transactions 28:6 (2000), pp. 654-658.	
	CX**	Poulos, A., "Very Long Chain Fatty Acids in Higher Animals - A Review", Lipids 30:1 (1995), pp. 1-14.	
	CY**	Huang, Y-S. et al., "Cloning of Δ12- and Δ6-Desaturases from *Mortierella alpina* and Recombinant Production of γ-Linolenic Acid in *Saccharomyces cerevisiae*", Lipids 34:7 (1999), pp. 649-659.	
	CZ**	Tocher, D.R. et al., "Recent Advances in the Biochemistry and Molecular Biology of Fatty Acyl Desaturases", Prog. Lipid Res. 37:2/3 (1998), pp. 73-117.	
	CA1**	Horrocks, L.A. et al., "Health Benefits of Docosahexaenoic Acid (DHA)", Pharmacological Research 40:3 (1999), pp. 211-225.	
	CB1**	McKeon, T. et al., "Stearoyl-Acyl Carrier Protein Desaturase from Safflower Seeds", in Methods in Enzymology, Vol. 71, Part C: Lipids, Editor: J. Lowenstein (1981), New York, pp. 275-281.	
	CC1**	Takeyama, H. et al., "Expression of the Eicosapentaenoic Acid Synthesis Gene Cluster from *Shewanella* sp. in a Transgenic Marine Cyanobacterium, *Synechococcus* sp.", Microbiology 143 (1997), pp. 2725-2731.	
	CD1**	Murphy, D.J. et al., "Biosynthesis, Targeting and Processing of Oleosin-like Proteins, Which are Major Pollen Coat Components in *Brassica napus*", Plant Journal 13:1 (1998), pp. 1-16.	
	CE1**	Wang, X.-M. et al., "Biosynthesis and Regulation of Linolenic Acid in Higher Plants", Physiol. Biochem. 26:6 (1988), pp. 777-792.	
	CF1**	Zank, T.K. et al., "Cloning and Functional Characterisation of an Enzyme Involved in the Elongation of Δ6-polyunsaturated Fatty Acids from the Moss *Physcomitrella patens*", The Plant Journal 31:3 (2002), pp. 255-268.	
	CG1**	Millar, A.A. et al., "*CUT1*, an Arabidopsis Gene Required for Cuticular Wax Biosynthesis and Pollen Fertility, Encodes a Very-Long-Chain Fatty Acid Condensing Enzyme", The Plant Cell 11 (1999), pp. 825-838.	
	CH1**	Calder, P.C., "Dietary Modification of Inflammation with Lipids", Proceedings of the Nutrition Society 61 (2002), pp. 345-358.	
	CI1**	Kunau, W.-H., et al., "β-oxidation of Fatty Acids in Mitochondria, Peroxisomes, and Bacteria: A Century of Continued Progress", Prog. Lipid Res. 34:4 (1995), pp. 267-342.	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	06/24/2017

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 5 of 6

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	CJ1**	Beaudoin, F. et al., "Heterologous Reconstitution in Yeast of the Polyunsaturated Fatty Acid Biosynthetic Pathway", Proceedings of the National Academy of Sciences of the United States of America 97:12 (2000), pp. 6421-6426.	
	CK1**	Ohlrogge, J. et al., "Lipid Biosynthesis", The Plant Cell 7 (1995), pp. 957-970.	
	CL1**	Millar, A.A. et al., "Very-long-chain Fatty Acid Biosynthesis is Controlled through the Expression and Specificity of the Condensing Enzyme", The Plant Journal 12:1 (1997), pp. 121-131.	
	CM1**	Shimokawa, H., "Beneficial Effects of Eicosapentaenoic Acid on Endothelial Vasodilator Functions in Animals and Humans", World Rev. Nutr. Diet 88 (2001), pp. 100-108.	
	CN1**	Chalova, L. I., et al. "The Composition of Lipids of Phytophthora infestans and Their Ability to Induce Potato Phytoalexin Accumulation". Biokhimiya, 1987, Vol. 52, No. 9, pp. 1445-1453; also see Database BIOSIS, Abstract No. PREV198885045135.	
	CO1**	Abbadi, A. et al., "Biosynthesis of Very-Long-Chain Polyunsaturated Fatty Acids in Transgenic Oilseeds: Constraints on Their Accumulation", The Plant Cell 16 (2004), pp. 2734-2748.	
	CP1**	"MY-26-A-10 PinfestansMY Phytophthora infestans cDNA, mRNA sequence." Database EMBL, Accession No. BE777235, September 21, 2000.	
	CQ1**	Domergue, F. et al., "Cloning and Functional Characterization of *Phaeodactylum tricornutum* Front-End Desaturases Involved in Eicosapentaenoic Acid Biosynthesis", Eur. J. Biochem. 269 (2002), pp. 4105-4113.	
	CR1**	Kamoun, S. et al., "Initial Assessment of Gene Diversity for the Oomycete Pathogen *Phytophthora infestans* Based on Expressed Sequences", Fungal Genetics and Biology 28 (1999), pp. 94-106.	
	CS1**	Khozin, I. et al., "Elucidation of the Biosynthesis of Eicosapentaenoic Acid in the Microalga *Porphyridium cruentum*", Plant Physiol. 114 (1997), pp. 223-230.	
	CT1**	Pereira, S.L. et al., "A Novel ω3-Fatty Acid Desaturase Involved in the Biosynthesis of Eicosapentaenoic Acid", Biochem. J. 378 (2004), pp. 665-671.	
	CU1**	Pereira, S.L. et al., "Recent Advances in the Study of Fatty Acid Desaturases from Animals and Lower Eukaryotes", Prostaglandins, Leukotrienes and Essential Fatty Acids 68 (2003), pp. 97-106.	
	CV1**	Spychalla, J.P. et al., "Identification of an Animal ω-3 Fatty Acid Desaturase by Heterologous Expression in *Arabidopsis*", Proc. Natl. Acad. Sci. USA 94 (1997), pp. 1142-1147.	
	CW1**	KAJIKAWA, M., et al., "Isolation and Functional Characterization of Fatty Acid Δ5-Elongase Gene from the Liverwort *Marchantia polymorpha* L.", FEBS Letters, 2006, Vol. 580, pp. 149-154.	
	CX1**	ROBERT, S. S., et al., "Isolation and Characterisation of a Δ5-Fatty Acid Elongase from the Marine Microalga *Pavlova salina*", Mar. Biotechnol., 2009, Vol. 11, pp. 410-418.	
	CY1**	PEREIRA, S. L., et al., "Identification of Two Novel Microalgal Enzymes Involved in the Conversion of the ω3-Fatty Acid, Eicosapentaenoic Acid, into Docosahexaenoic Acid", Biochem. J., 2004, Vol. 384, pp. 357-366.	
	CZ1**	LEONARD, A. E., et al., "Elongation of Long-Chain Fatty Acids", Progress in Lipid Research, 2004, Vol. 43, pp. 36-54.	
	CA2**	SPERLING, P., et al., "The Evolution of Desaturases", Prostaglandins, Leukotrienes and Essential Fatty Acids, 2003, Vol. 68, pp. 73-95.	
	CB2**	"LOC398440 Protein", UniProt Database Accession No. Q7ZXJ4, June 1, 2003.	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	06/24/2017

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	6	of	6

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrent Herewith
First Named Inventor	Petra Cirpus
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	074017-0013-01-US

	CC2**	DOMERGUE, F., et al., "New Insight into *Phaeodactylum tricornutum* Fatty Acid Metabolism. Cloning and Functional Characterization of Plastidial and Microsomal Δ12-Fatty Acid Desaturases", Plant Physiology, 2003, Vol. 131, pp. 1648-1660.	
	CD2**	WU, G., et al., "Stepwise Engineering to Produce High Yields of Very Long-Chain Polyunsaturated Fatty Acids in Plants", Nature Biotechnology, 2005, Vol. 23, pp. 1013-1017.	
	CE2**	NAKAMURA, M. T., et al., "Structure, Function, and Dietary Regulation of Δ6, Δ5, and Δ9 Desaturases", Annu. Rev. Nutr., 2004, Vol. 24, pp. 345-376.	
	CF2**	"P. patens Delta6 Elongase SEQ ID 29", GeneSeq Database Accession No. ABG73608, March 25, 2003.	
	CG2**	"Subname: Full = Polyunsaturated Fatty Acid Elongase elvol5a", UniProt Database Accession No. Q8AWE7, October 25, 2005.	
	CH2**	"Polyunsaturated Fatty Acid Elongase (ELOVL Family Member 5, Elongation of Long Chain Fatty Acids) (YEAST)", UniProt Database Accession No. Q8AX86, March 1, 2003.	
	CI2**	"633167 NCCCWA 1RT Oncorhynchus Mykiss cDNA Clone 1RT126D03_B_B02 5', mRNA Sequence", EMBL Database Accession No. CA360014, November 7, 2002.	
	CJ2**	HUANG, Y.-S., et al., "Enzymes for Transgenic Biosynthesis of Long-Chain Polyunsaturated Fatty Acids", Biochimie, 2004, Vol. 86, No. 11, pp. 793-798.	
	CK2**	"Physcomitrella patens Desaturase Encoding cDNA SEQ ID NO 7", GeneSeq Database Accession No. ABV74260, March 28, 2003.	
	CL2**	"Phaeodactylum tricornutum Desaturase Encoding cDNA SEQ ID NO 11", GeneSeq Database Accession No. ABV74262, March 28, 2003.	
	CM2**	Sprecher, H. "Metabolism of Highly Unsaturated n-3 and n-6 Fatty Acids", Biochimica et Biophysica Acta, 2000, Vol. 1486, pp. 219-231.	
	CN2**	"Nouveau Dictionnaire des Huiles Végétales: Compositions en Acides Gras", Ucciani E., Ed. Technique & Documentation - Lavoisier, 1995, ISBN: 2-7430-0009-0, pp. 577, 578 and 582.	
	CO2**	"Phaeodactylum tricornutum Elongase Encoding cDNA SEQ ID NO 9",GeneSeq Database Accession No. ABV74261, March 28, 2003.	
	CP2**	Bork, P., et al., "Go Hunting in Sequence Databases but Watch Out for the Traps", Trends in Genet. 12:10 (1996), pp. 425-427.	
	CQ2**	Broun, P., et al., "Catalytic Plasticity of Fatty Acid Modification Enzymes Underlying Chemical Diversity of Plant Lipids", Science 282:5392 (1998), pp. 1315-1317.	
	CR2**	van de Loo, F. J., et al., "An Oleate 12-Hydroxylase from *Ricinus communis* L. is a Fatty Acyl Desaturase Homolog", Proc. Natl. Acad. Sci. U S A 92:15 (1995), pp. 6743-6747.	
	CS2**	Doerks, T., et al., "Protein Annotation: Detective Work for Function Prediction", Trends in Genet. 14:6 (1998), pp. 248-250.	
	CT2**	Brenner, S. E., "Errors in Genome Annotation", Trends in Genet. 15:4 (1999), pp. 132-133.	
	CU2**	Yu, Z., et al., "Study on Nutritional Function of Polyunsaturated Fatty Acid", China Feed, 2003, Issue 24, pp. 21-23.	
	CV2**	Qi, B., et al., "Production of Very Long Chain Polyunsaturated Omega-3 and Omega-6 Fatty Acids in Plants", Nature Biotechnology, 2004, Vol. 22, No. 6, pp. 739-745.	
	CW2**	Robert, S. S., "Production of Eicosapentaenoic and Docosahexaenoic Acid-Containing Oils in Transgenic Land Plants for Human and Aquaculture Nutrition, Marine Biotechnology, 2006, 8: 103-109.	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	06/24/2017

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	Art Unit
	HOPE ROBINSON	1652

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
NONE		6/21/17	HAR

SEARCH NOTES		
Search Notes	Date	Examiner
WEST	6/21/17	HAR
Palm Expo inventor names searched	6/21/17	HAR
NPL search (DHA, transgeic Brassica plant, oils, lipids and fatty acids, LCPUFA, triacylglycerides)	6/21/17	HAR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

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CONFIRMATION NO. 4050

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/256,914	09/06/2016	514	1652	074017-0013-01-US

APPLICANTS

BASF Plant Science GmbH, Ludwigshafen, GERMANY;

INVENTORS

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Bifang Cheng, Saskatoon, CANADA;
Martin Truksa, Saskatoon, CANADA;
Tom Wetjen, Mannheim, GERMANY;

**** CONTINUING DATA ***************************

This application is a CON of 12/280,090 08/20/2008
which is a 371 of PCT/EP2007/051675 02/21/2007

**** FOREIGN APPLICATIONS ***************************

EUROPEAN PATENT OFFICE (EPO) 06120309.7 09/07/2006
GERMANY 102006008030.0 02/21/2006

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

09/13/2016

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☑ Yes ☐ No	☑ Yes ☐ No	/HOPE A ROBINSON/ Examiner's Signature	☐ Initials	GERMANY	0	22	2

ADDRESS

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621
UNITED STATES

TITLE

METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

FILING FEE RECEIVED		
1840	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

BIB (Rev. 05/07).

WEST Search History for Application 15256914

Creation Date: 2017062115:11

Interference Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
(cirpus.in. and qiu.in.) and ((oils, SPACE~lipids, SPACE~fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
Bauer.in.	PGPB, USPT	11179	OR	YES		06-21-2017
(Bauer.in.) and (cirpus.in. and qiu.in. and (oils, SPACE~lipids, SPACE~fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017

Prior Art Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
9458436.pn.	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (oils)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (brassica plant)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (4 percent DHA)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (sn-1)	USPT	0	OR	YES		06-21-2017
(9458436.pn.) and (sn-2)	USPT	0	OR	YES		06-21-2017
(9458436.pn.) and (sn-2) position	USPT	3490632	OR	YES		06-21-2017
(9458436.pn.) and (sn-2 position)	USPT	1	OR	YES		06-21-2017
(oils, lipids, fatty acids and transgenic Brassica plant)	PGPB, USPT	2231550	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant)) and (DHA)	PGPB, USPT	16649	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA)) and	PGPB, USPT	16539	OR	YES		06-21-2017

(polyunsaturated fatty acids)						
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids)) and (sn-1 or sn-2 or sn-3 position)	PGPB, USPT	8846	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position)) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.)	PGPB, USPT	1564	OR	YES		06-21-2017
qiu.in.	PGPB, USPT	5533	OR	YES		06-21-2017
cirpus.in.	PGPB, USPT	60	OR	YES		06-21-2017
(cirpus.in.) and (qiu.in.)	PGPB, USPT	12	OR	YES		06-21-2017
(cirpus.in. and qiu.in.) and ((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
20160369290	PGPB, USPT	1	OR	YES		06-21-2017
38137303.FMID.	USPT, FPRS, PGPB	9	OR	YES		06-21-2017

Docket No.: 074017-0013-01-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: Hope A. Robinson

AMENDMENT AND REPLY UNDER 37 CFR §1.111

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated July 21, 2017, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 4 of this paper.

Remarks begin on page 8 of this paper.

An **Appendix** including a clean copy of Substitute Specification is attached following page 11 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the specification filed on September 6, 2016 with the Substitute Specification submitted herewith.

Attachment: Clean Copy of Substitute Specification

Please delete the title of invention as appeared in the Substitute Specification submitted herewith with the following new title:

"Oils, lipids and fatty acids produced in transgenic *Brassica* plant"

In the Substitute Specification submitted herewith at page 9, line 18, please replace the paragraph starting with "In addition" with the following amended paragraph:

In addition, different organisms prefer different codons. For this reason, for example, the expression of a recombinant DNA derived from a mammalian cell frequently proceeds only suboptimally in *Escherichia coli* (*E. coli*) cells. It is therefore possible in some cases to increase expression by replacing rarely used codons with frequently used codons. Without wishing to be bound to one theory, it is assumed that the codon-optimized DNA sequences make more efficient translation possible, and the mRNAs formed therefrom possibly have a greater half-life in the cell and therefore are available more frequently for translation. From what has been said above, it follows that codon optimization is necessary only if the organism in which the nucleic acid sequence is to be expressed differs from the organism from which the nucleic acid sequence is originally derived.

In the Substitute Specification submitted herewith at page 9, line 28, please replace the paragraph starting with "For many organisms" with the following amended paragraph:

For many organisms of which the DNA sequence of a relatively large number of genes is known there are tables from which the frequency of use of particular codons in the respective organism can be taken. It is possible with the aid of these tables to translate protein sequences with relatively high accuracy back into a DNA sequence which comprises the codons preferred in the respective organism for the various amino acids of the protein. Tables on codon usage can be found inter alia at the following Internet address: ~~www.~~kazusa.or.ip/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelechon (Software Leto) or Geneart (Software GeneOptimizer).

In the Substitute Specification submitted herewith at page 12, line 29, please replace the paragraph starting with "Further suitable" with the following amended paragraph:

Further suitable nucleic acid sequences can be found by the skilled worker from the literature or the well-known gene libraries such as, for example, ~~www.~~ncbi.nlm.nih.gov.

In the Substitute Specification submitted herewith at page 80, line 24, please replace the paragraph starting with "It was possible" with the following amended paragraph:

It was possible to find VLCPUFA (very long chain polyunsaturated fatty acid) in these lipids too, with an accumulation of EPA in the sn-2 position being observed. DHA was to be found only in the digalactodiacylglycerols (DGDG) and was undetectable in the monogalactodiacylglycerols (MGDG) (Table 5). The distribution of VLCPUFA in galactolipids, a compartment in which these fatty acids were not expected, shows the dynamics of the synthesis and the later transformation. VLCPUFA in polar lipids are of particular nutritional value because they can be absorbed better in the intestines of mammals than the neutral lipids.

AMENDMENTS TO THE CLAIMS

Listing of Claims:

1. (Currently amended) Oils, lipids and/or fatty acids produced by a transgenic ~~Brassica~~ *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant, wherein said polyunsaturated fatty acids comprise at least 20% by weight of eicosapentaenoic acid (EPA), at least 2% by weight of docosapentaenoic acid (DPA), and/or at least 4% by weight of docosahexaenoic acid (DHA) based on the total fatty acids in the transgenic plant in form of triacylglycerides.

2. (Original) The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-1, sn-2 or sn-3 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 1.5% by weight of DPA is present in the sn-1, sn-2 or sn-3 position; and/or

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-1, sn-2 or sn-3 position.

3. (Original) The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-2 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 3% by weight of DPA is present in the sn-2 position; and/or

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-2 position.

4. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise:

a) at least 20% by weight of EPA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides;

b) at least 20% by weight of EPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides; or

c) at least 2% by weight of DPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

5. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

6. (Withdrawn) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

7. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

8. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

9. (Currently amended) Oils, lipids and/or fatty acids produced by a transgenic ~~Brassica~~ *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

10. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant.

11. (Withdrawn) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 20% by weight of EPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

12. (Withdrawn) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated

fatty acids comprise at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

13. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

14. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

15. (Withdrawn) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

16. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

17. (Withdrawn; currently amended) A method for producing oils, lipids and/or fatty acids of claim 1, comprising expressing in a ~~Brassica~~ *Brassica* plant a nucleic acid encoding a Δ6-desaturase, a nucleic acid encoding a Δ5-desaturase, a nucleic acid encoding a Δ6-elongase, a nucleic acid encoding a ω3-desaturase, a nucleic acid encoding a Δ5-elongase, and a nucleic acid encoding a Δ4-desaturase, wherein said nucleic acid encoding a Δ5-elongase is codon-optimized by adapting to the codon usage of ~~Brassica~~ *Brassica*.

18. (Withdrawn; currently amended) The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a nucleotide sequence having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 64, and wherein said nucleotide sequence is obtained by adapting at least 30% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of ~~Brassica~~ *Brassica*.

19. (Withdrawn) The method of claim 18, wherein said nucleotide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 64, or wherein said nucleotide

sequence encodes a polypeptide having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65.

20. (Withdrawn) The method of claim 18, wherein said nucleotide sequence is adapted taking into account the natural frequency of individual codons.

21. (Withdrawn; currently amended) The method of claim 18, wherein said nucleotide sequence is obtained by adapting at least 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of ~~Brassica~~ *Brassica*.

22. (Withdrawn; currently amended) The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a translated section coding for a protein having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65, and wherein the translated section comprises a nucleotide sequence obtained by adapting at least 30% or 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of ~~Brassica~~ *Brassica*.

23. (Previously presented) The oils, lipids and/or fatty acids of claim 1, comprising:

a) at least 4% by weight of DHA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant;

b) at least 4% by weight of DHA and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant; or

c) at least 4% by weight of DHA, at least 2% by weight of DPA, and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant.

REMARKS

After entry of this amendment, claims 1-23 are pending, of which claims 6, 11, 12, 15, 17-22 are withdrawn. Claims 1 and 9 have been amended without prejudice or disclaimer. Support for the amendment is found *inter alia* in the original claims. No new matter has been added.

Withdrawn claims 17, 18, 21 and 22 have also been amended without prejudice or disclaimer to correct the informalities identified by the Examiner. Support for the amendment is found *inter alia* in the original claims. No new matter has been added.

Specification

The title of the invention is objected to as being allegedly not descriptive. In response, the title of the invention has been amended as suggested by the Examiner. Applicant believes that the objection is rendered moot and should be withdrawn.

The specification is objected for not italicizing the organism names. In response, Applicant submits herewith a Substitute Specification in compliance with 37 CFR §§ 1.121 and 1.125 to replace the specification as originally filed on September 6, 2016. All the organism names in the Substitute Specification submitted herewith have been italicized as required by the Examiner. Because the only changes made in the Substitute Specification submitted herewith are to italicize the organism names and no other substantive changes were made, no mark-up copy of the Substitute Specification is provided herewith. Applicant submits that no new matter is involved with the Substitute Specification. Entry of the Substitute Specification is respectfully requested.

The specification is further objected to for not spelling out meanings of acronyms at their first occurrence. In response, the Substitute Specification submitted herewith has been amended to spell out meanings of acronyms at their first occurrence. No new matter has been added.

Reconsideration and withdrawal of the objections is respectfully requested.

Information Disclosure Statement

Applicant thanks the Examiner for entering and considering the information disclosure statements previously submitted. Applicant notes that the Examiner has indicated that there is

one reference being lined through because of the improper citation. Office Action at page 4. However, Applicant was not able to find such a lined-through reference on the initialed forms attached to the Office Action. Applicant respectfully requests the Examiner clarify which reference containing improper citation.

Claim Objection

Claims 1 and 9 are objected to for not italicizing the organism names. In response, claims 1 and 9 have been amended without prejudice or disclaimer to correct the informalities identified by the Examiner. Applicant believes that the objection is rendered moot and should be withdrawn.

Claim Rejection – 35 U.S.C. § 102

Claims 1-5, 7-10, 13, 14, 16 and 23 are rejected under pre-AIA 35 U.S.C. §102(e) as being allegedly anticipated by Cirpus *et al.* (hereinafter "Cirpus"). Applicant strongly disagrees and traverses the rejection for the following reasons.

As an initial matter, it is noted that Cirpus was issued from an application that was filed as the national stage entry of the corresponding PCT application published as WO 2005/083093. Because WO 2005/083093 was not published in English, neither Cirpus nor WO 2005/083093 has a 102(e) date for prior art purposes.

Nevertheless, Applicant notes that WO 2005/083093 was published on September 9, 2005, which makes this reference potentially applicable under Section 102 against the present claims. Because Cirpus is the national stage entry of WO 2005/083093, these two references share the same disclosure. Accordingly, Applicant addresses this rejection as it were made based on WO 2005/083093 instead of Cirpus.

The Examiner asserts that Cirpus discloses the production of polyunsaturated fatty acids in the seeds of transgenic plants by expressing nucleic acid sequences which increase the content of polyunsaturated fatty acids as wells as oils, lipids and/or fatty acids with an elevated content of polyunsaturated fatty acids. The Examiner further alleges that the percentage for DHA disclosed in Cirpus corresponds to that recited in the claims. Applicant strongly disagrees.

The present claims are directed to oils, lipids and/or fatty acids which comprise an EPA (eicosapentaenoic acid) content of at least 20%, a DHA (docosahexaenoic acid) content of at

least 4%, and/or a DPA (docosapentaenoic acid) content of at least 2% by weight based on the total fatty acids in the transgenic plant.

In contrast, WO 2005/083093 discloses products comprising at least 16% by weight of EPA (see e.g., page 26, lines 14-18 of WO 2005/083093) and at least 0.35% by weight of DHA (see e.g., page 26, lines 20-24 of WO 2005/083093). WO 2005/083093 does not disclose any DPA content.

Moreover, according to Tables 22 and 23 on page 158 of WO 2005/083093, transgenic seeds showed an EPA content of 0.2 to 2.6% based on the total fatty acids. Table 3 on pages 161 and 162 of WO 2005/083093 shows that seeds from transgenic *B. juncea* lines had an EPA content of 0.94 to 1.21% and Table 4 on page 163 shows that transgenic *B. juncea* lines had an EPA content of 0.8 to 2.6%. Hence, the EPA content disclosed in WO 2005/083093 is considerably lower than the EPA content required by the present claims.

Additionally, Table 24 on page 165 of WO 2005/083093 shows that transgenic seeds had an EPA content of 8.65 to 9.43%, a DPA content of 0.19 to 0.24% and a DHA content of 0.23 to 0.40%.

Clearly, WO 2005/083093 does not disclose oils, lipids and/or fatty acids having the specific fatty acid contents as required by the present claims.

The Examiner also contends that the content of polyunsaturated ω3 fatty acids of at least 54% as recited in claim 9 is obvious based on Cirpus. Applicant respectfully disagrees and wishes to note that, according to Table 24 on page 165 of WO 2005/083093, the sum of all polyunsaturated ω3 fatty acids is only 14.58 to 15.72 %. Thus, WO 2005/083093 clearly does not disclose that the content of polyunsaturated ω3 fatty acids as recited in claim 9 would have been obtained in the disclosed transgenic *Brassica* plants as alleged by the Examiner. Accordingly, one skilled in the art would not have been motivated to use transgenic Brassica plants disclosed in WO 2005/083093 to produce oils, lipids and fatty acids having the specific content of polyunsaturated ω3 fatty acids as recited in claim 9.

For at least the above reasons, Applicant submits that neither Cirpus nor WO 2005/083093 anticipates the claims as now presented. Reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the above remarks and further in view of the present claims, Applicant respectfully requests withdrawal of the rejection and allowance of the claims. If any outstanding issues remain, the Examiner is invited to telephone the undersigned at the number given below.

Applicant reserves all rights to pursue the non-elected and/or cancelled subject matter in one or more continuing applications.

This response is filed within the three-month response period from the mailing of the Office Communication, to and include October 23, 2017 pursuant to 37 CFR § 1.7(a). No fee is believed due. However, if a fee is due, please charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US, from which the undersigned is authorized to draw.

Respectfully submitted,

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#89,696,397

Method for producing polyunsaturated fatty acids

RELATED APPLICATIONS

This application is a continuation of patent application Serial No. 12/280,090 filed August 20, 2008, which is a national stage application (under 35 U.S.C. § 371) of PCT/EP2007/051675, filed February 21, 2007, which claims benefit of German application 10 2006 008 030.0, filed February 21, 2006 and European application 06120309.7, filed September 7, 2006. The entire content of each aforementioned application is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING

The Sequence Listing associated with this application is filed in electronic format *via* EFS-Web and hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is Sequence_Listing_074017_0013_01. The size of the text file is 730 KB, and the text file was created on September 2, 2016.

The present invention relates to a process for the production of eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in transgenic plants, providing in the plant at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity, where the nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified by comparison with the nucleic acid sequence in the organism from which the sequence is derived in that it is adapted to the codon usage in one or more plant species.

In a preferred embodiment there is additionally provision of further nucleic acid sequences which code for a polypeptide having the activity of an ω3-desaturase and/or of a Δ4-desaturase in the plant.

In a further preferred embodiment there is provision of further nucleic acid sequences which code for acyl-CoA dehydrogenase(s), acyl-ACP (acyl carrier protein) desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyl transferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A

carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) in the plant.

The invention furthermore relates to recombinant nucleic acid molecules comprising at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species.

A further part of the invention relates to oils, lipids and/or fatty acids which have been produced by the process according to the invention, and to their use.

Finally, the invention also relates to transgenic plants which have been produced by the process of the invention or which comprise a recombinant nucleic acid molecule of the invention, and to the use thereof as foodstuffs or feedstuffs.

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3-phosphate, and the addition or modification of a polar head group. Usual lipids which are used in membranes comprise phospholipids, glycolipids, sphingolipids and phosphoglycerides. Fatty acid synthesis starts with the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase or into acetyl-ACP by acetyl transacylase. After condensation reaction, these two product molecules together form acetoacetyl-ACP, which is converted via a series of condensation, reduction and dehydration reactions so that a saturated fatty acid molecule with the desired chain length is obtained. The production of the unsaturated fatty acids from these molecules is catalyzed by specific desaturases, either aerobically by means of molecular oxygen or anaerobically (regarding the fatty acid synthesis in microorganisms, see F.C. Neidhardt et al. (1996) E. coli and Salmonella. ASM Press: Washington, D.C., p. 612-636 and references cited therein; Lengeler et al. (Ed.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, and the references therein, and Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 and the references therein). To undergo the further elongation steps, the resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This is made possibly by acyl-CoA:lysophospho-

lipid acyltransferases. Moreover, these enzymes are capable of transferring the elongated fatty acids from the CoA esters back to the phospholipids. If appropriate, this reaction sequence can be followed repeatedly.

Furthermore, fatty acids must subsequently be transported to various modification sites and incorporated into the triacylglycerol storage lipid. A further important step during lipid synthesis is the transfer of fatty acids to the polar head groups, for example by glycerol fatty acid acyltransferase (see Frentzen, 1998, Lipid, 100(4-5):161-166).

An overview of the biosynthesis of fatty acids in plants, desaturation, the lipid metabolism and the membrane transport of lipidic compounds, beta-oxidation, the modification of fatty acids, cofactors and the storage and assembly of triacylglycerol, including the references is given by the following papers: Kinney (1997) Genetic Engineering, Ed.: JK Setlow, 19:149-166; Ohlrogge and Browse (1995) Plant Cell 7:957-970; Shanklin and Cahoon (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker (1996) Genetic Engeneering, Ed.: JK Setlow, 18:111-13; Gerhardt (1992) Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl (1995) Biochim. Biophys Acta 1256:181-186; Kunau et al. (1995) Prog. Lipid Res. 34:267-342; Stymne et al. (1993) in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Ed.: Murata und Somerville, Rockville, American Society of Plant Physiologists, 150-158; Murphy & Ross (1998) Plant Journal. 13(1):1-16.

Depending on the desaturation pattern, two large classes of polyunsaturated fatty acids, the ω6 and the ω3 fatty acids, which differ with regard to their metabolism and their function, can be distinguished.

In the text which follows, polyunsaturated fatty acids are referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (poly unsaturated fatty acids, PUFA, long chain poly unsaturated fatty acids, LCPUFA).

The fatty acid linoleic acid ($18:2^{\Delta 9,12}$) acts as starting material for the ω6 metabolic pathway, while the ω3 pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed from linoleic acid by the activity of an ω3-desaturase (Tocher et al. (1998) Prog. Lipid Res. 37: 73-117; Domergue et al. (2002) Eur. J. Biochem. 269: 4105-4113).

Mammals, and thus also humans, have no corresponding desaturase activity (Δ12- and ω3-desaturase) for the formation of the starting materials and must therefore take up these fatty acids (essential fatty acids) via the food. Starting with these precursors, the physiologically

important polyunsaturated fatty acids arachidonic acid (= ARA, 20:4$^{\Delta 5,8,11,14}$), an ω6-fatty acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, 20:5$^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA, 22:6$^{\Delta 4,7,10,13,17,19}$) are synthesized via a sequence of desaturase and elongase reactions.

The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C_{20}- and C_{22}-PUFAs, respectively. This process proceeds via 4 steps. The first step is the condensation of malonyl-CoA onto the fatty acid acyl-CoA by ketoacyl-CoA synthase (KCS, hereinbelow referred to as elongase). This is followed by a reduction step (ketoacyl-CoA reductase, KCR), a dehydratation step (dehydratase) and a final reduction step (enoyl-CoA reductase). It has been postulated that the elongase activity affects the specificity and rate of the entire process (Millar and Kunst (1997) Plant Journal 12:121-131).

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and the pharmacological sector. Depending on whether they are free saturated or unsaturated fatty acids or else triacylglycerides with an elevated content of saturated or unsaturated fatty acids, they are suitable for very different applications. Thus, for example, lipids with unsaturated, specifically with polyunsaturated fatty acids, are preferred in human nutrition. The polyunsaturated ω3-fatty acids are supposed to have a positive effect on the cholesterol level in the blood and thus on the prevention of heart disease. The risk of heart disease, strokes or hypertension can be reduced markedly by adding these ω3-fatty acids to the food (Shimikawa (2001) World Rev. Nutr. Diet. 88: 100-108).

ω3-fatty acids also have a positive effect on inflammatory, specifically on chronically inflammatory, processes in association with immunological diseases such as rheumatoid arthritis (Calder (2002) Proc. Nutr. Soc. 61: 345-358; Cleland and James (2000) J. Rheumatol. 27: 2305-2307). They are therefore added to foodstuffs, specifically to dietetic foodstuffs, or are employed in medicaments. ω6-fatty acids such as arachidonic acid tend to have a negative effect in connection with these rheumatological diseases.

ω3- and ω6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG_2 series) which are formed from the ω6-fatty acids, generally promote inflammatory reactions, while eicosanoids (known as the PG_3 series) from ω3-fatty acids have little or no proinflammatory effect.

Polyunsaturated long-chain ω3-fatty acids such as eicosapentaenoic acid (= EPA, C20:5$^{\Delta5,8,11,14,17}$) or docosahexaenoic acid (= DHA, C22:6$^{\Delta4,7,10,13,16,19}$) are important components of human nutrition owing to their various roles in health aspects, including the development of the child brain, the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A (1995) Lipids 30:1-14; Horrocks, LA and Yeo YK (1999) Pharmacol Res 40:211-225).

Owing to the present-day composition of human food, an addition of polyunsaturated ω3-fatty acids, which are preferentially found in fish oils, to the food is particularly important. Thus, for example, polyunsaturated fatty acids such as docosahexaenoic acid (= DHA, C22:6$^{\Delta4,7,10,13,16,19}$) or eicosapentaenoic acid (= EPA, C20:5$^{\Delta5,8,11,14,17}$) are added to infant formula to improve the nutritional value. There is therefore a demand for the production of polyunsaturated long-chain fatty acids.

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella or Schizochytrium or from oil-producing plants such as soybeans, oilseed rape, and algae such as *Crypthecodinium* or *Phaeodactylum* and others, being obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, for example, fish. The free fatty acids are advantageously prepared by hydrolyzing the triacylglycerides. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (ARA, C20:4$^{\Delta5,8,11,14}$), dihomo-γ-linolenic acid (DHGL, C20:3$^{\Delta8,11,14}$) or docosapentaenoic acid (DPA, C22:5$^{\Delta7,10,13,16,19}$) are, however, not synthesized in oil crops such as oilseed rape, soybeans, sunflowers and safflower. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Owing to the positive characteristics of the polyunsaturated fatty acids, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of these fatty acids or triglycerides for the production of oils in various organisms with a modified content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describe a Δ9-desaturase. WO 93/11245 claims a Δ15-desaturase and WO 94/11516 a Δ12-desaturase. Further desaturates are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al. (1990) J. Biol. Chem., 265: 20144-20149, Wada et al. (1990) Nature 347: 200-203 or Huang et al. (1999)

Lipids 34: 649-659. However, the biochemical characterization of the various desaturases has been insufficient to date since the enzymes, being membrane-bound proteins, present great difficulty in their isolation and characterization (McKeon et al. (1981) Methods in Enzymol. 71: 12141-12147, Wang et al. (1988) Plant Physiol. Biochem., 26: 777-792).

As a rule, membrane-bound desaturases are characterized by being introduced into a suitable organism which is subsequently analyzed for enzyme activity by analyzing the starting materials and the products. Δ6-Desaturases are described in WO 93/06712, US 5,614,393, WO 96/21022, WO 00/21557 and WO 99/27111. The application of this enzyme for the production of fatty acids in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. The expression of various desaturases and the formation of polyunsaturated fatty acids is also described and claimed in WO 99/64616 or WO 98/46776. As regards the expression efficacy of desaturases and its effect on the formation of polyunsaturated fatty acids, it must be noted that the expression of a single desaturase as described to date has only resulted in low contents of unsaturated fatty acids/lipids such as, for example, γ-linolenic acid and stearidonic acid.

There have been a number of attempts in the past to obtain elongase genes. Millar and Kunst (1997) Plant Journal 12:121-131 and Millar et al. (1999) Plant Cell 11:825-838 describe the characterization of plant elongases for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the formation of waxes in plants (C_{28}-C_{32}). The synthesis of arachidonic acid and EPA is described, for example, in WO 01/59128, WO 00/12720, WO 02/077213 and WO 02/08401. The synthesis of polyunsaturated C24-fatty acids is described, for example, in Tvrdik et al. (2000) J. Cell Biol. 149:707-718 or in WO 02/44320.

Especially suitable microorganisms for the production of PUFAs are microalgae such as *Phaeodactylum tricornutum*, *Porphiridium* species, *Thraustochytrium* species, *Schizochytrium* species or *Crypthecodinium* species, ciliates such as *Stylonychia* or *Colpidium*, fungi such as *Mortierella*, *Entomophthora* or *Mucor* and/or mosses such as *Physcomitrella*, *Ceratodon* and *Marchantia* (R. Vazhappilly & F. Chen (1998) Botanica Marina 41: 553-558; K. Totani & K. Oba (1987) Lipids 22: 1060-1062; M. Akimoto et al. (1998) Appl. Biochemistry and Biotechnology 73: 269-278). Strain selection has resulted in the development of a number of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFAs. However, the mutation and

selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult process. Moreover, only limited amounts of the desired polyunsaturated fatty acids such as DPA, EPA or ARA can be produced with the aid of the abovementioned microorganisms; in addition, they are generally obtained as fatty acid mixtures. This is why recombinant methods are preferred whenever possible.

Higher plants comprise polyunsaturated fatty acids such as linoleic acid (C18:2) and linolenic acid (C18:3). ARA, EPA and DHA are found not at all in the seed oil of higher plants, or only in miniscule amounts (E. Ucciani: Nouveau Dictionnaire des Huiles Végétales [New Dictionary of the Vegetable Oils]. Technique & Documentation – Lavoisier, 1995. ISBN: 2-7430-0009-0). However, the production of LCPUFAs in higher plants, preferably in oil crops such as oilseed rape, linseed, sunflowers and soybeans, would be advantageous since large amounts of high-quality LCPUFAs for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically. To this end, it is advantageous to introduce, into oilseeds, genes which encode enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. These genes encode for example Δ6-desaturases, Δ6-elongases, Δ5-desaturases or Δ4-desaturases. These genes can advantageously be isolated from microorganisms and lower plants which produce LCPUFAs and incorporate them in the membranes or triacylglycerides. Thus, it has already been possible to isolate Δ6-desaturase genes from the moss *Physcomitrella patens* and Δ6-elongase genes from *P. patens* and from the nematode *C. elegans*.

Transgenic plants which comprise and express genes encoding LCPUFA biosynthesis enzymes and which, as a consequence, produce LCPUFAs have been described, for example, in DE-A-102 19 203 (process for the production of polyunsaturated fatty acids in plants). However, these plants produce LCPUFAs in amounts which require further optimization for processing the oils which are present in the plants. Thus, the ARA content in the plants described in DE-A-102 19 203 is only 0.4 to 2% and the EPA content only 0.5 to 1%, in each case based on the total lipid content of the plant.

To make possible the fortification of food and of feed with polyunsaturated, long-chain fatty acids, there is therefore a great need for a simple, inexpensive process for the production of polyunsaturated, long-chain fatty acids, specifically in plant systems.

One object of the invention is therefore to provide a process with which long-chain polyunsaturated fatty acids, especially eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid can be produced in large quantities and inexpensively in transgenic plants.

It has now surprisingly been found that the yield of long-chain polyunsaturated fatty acids, especially eicosapentaenoic, docosapentaenoic acid and/or docosahexaenoic acid, can be increased by expressing an optimized Δ5-elongase sequence in transgenic plants.

The PUFAs produced by the process of the invention comprise a group of molecules which higher animals are no longer able to synthesize and thus must consume, or which higher animals are no longer able to produce themselves in sufficient amounts and thus must consume additional amounts thereof, although they can easily be synthesized by other organisms such as bacteria.

Accordingly, the object of the invention is achieved by the process of the invention for producing eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in a transgenic plant, comprising the provision in the plant of at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity,

where the nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified by comparison with the nucleic acid sequence in the organism from which the sequence is derived in that it is adapted to the codon usage in one or more plant species. To produce DHA it is additionally necessary to provide at least one nucleic acid sequence which codes for a polypeptide having a Δ4-desaturase activity in the plant.

The “provision in the plant” means in the context of the present invention that measures are taken so that the nucleic acid sequences coding for a polypeptide having a Δ6-desaturase activity, a polypeptide having a Δ6-elongase activity, a polypeptide having a Δ5-desaturase activity and a polypeptide having a Δ5-elongase activity are present together in one plant. The “provision in the plant” thus comprises the introduction of the nucleic acid sequences into the plant both by transformation of a plant with one or more recombinant nucleic acid

molecules which comprise said nucleic acid sequences, and by crossing suitable parent plants which comprise one or more of said nucleic acid sequences.

The nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified according to the invention by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species. This means that the nucleic acid sequence has been specifically optimized for the purpose of the invention without the amino acid sequence encoded by the nucleic acid sequence having been altered thereby.

The genetic code is redundant because it uses 61 codons in order to specify 20 amino acids. Therefore, most of the 20 proteinogenic amino acids are therefore encoded by a plurality of triplets (codons). The synonymous codons which specify an individual amino acid are, however, not used with the same frequency in a particular organism; on the contrary there are preferred codons which are frequently used, and codons which are used more rarely. These differences in codon usage are attributed to selective evolutionary pressures and especially the efficiency of translation. One reason for the lower translation efficiency of rarely occurring codons might be that the corresponding aminoacyl-tRNA pools are exhausted and thus no longer available for protein synthesis.

In addition, different organisms prefer different codons. For this reason, for example, the expression of a recombinant DNA derived from a mammalian cell frequently proceeds only suboptimally in *E. coli* cells. It is therefore possible in some cases to increase expression by replacing rarely used codons with frequently used codons. Without wishing to be bound to one theory, it is assumed that the codon-optimized DNA sequences make more efficient translation possible, and the mRNAs formed therefrom possibly have a greater half-life in the cell and therefore are available more frequently for translation. From what has been said above, it follows that codon optimization is necessary only if the organism in which the nucleic acid sequence is to be expressed differs from the organism from which the nucleic acid sequence is originally derived.

For many organisms of which the DNA sequence of a relatively large number of genes is known there are tables from which the frequency of use of particular codons in the respective organism can be taken. It is possible with the aid of these tables to translate protein sequences with relatively high accuracy back into a DNA sequence which comprises the codons

preferred in the respective organism for the various amino acids of the protein. Tables on codon usage can be found inter alia at the following Internet address:

www.kazusa.or.ip/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelechon (Software Leto) or Geneart (Software GeneOptimizer).

Adaptation of the sequences to the codon usage in a particular organism can take place with the aid of various criteria. On the one hand, it is possible to use for a particular amino acid always the codon which occurs most frequently in the selected organism but, on the other hand, the natural frequency of the various codons can also be taken into account, so that all the codons for a particular amino acid are incorporated into the optimized sequence according to their natural frequency. Selection of the position at which a particular base triplet is used can take place at random in this case. The DNA sequence was adapted according to the invention taking account of the natural frequency of individual codons, it also being suitable to use the codons occurring most frequently in the selected organism.

It is particularly preferred for a nucleic acid sequence from *Ostreococcus tauri* which codes for a polypeptide having a Δ5-elongase activity, such as, for example, the polypeptide depicted in SEQ ID NO: 110, to be adapted at least to the codon usage in oilseed rape, soybean and/or flax. The nucleic acid sequence originally derived from *Ostreococcus tauri* is preferably the sequence depicted in SEQ ID NO: 109. The DNA sequence coding for the Δ5-elongase is adapted in at least 20% of the positions, preferably in at least 30% of the positions, particularly preferably in at least 40% of the positions and most preferably in at least 50% of the positions to the codon usage in oilseed rape, soybean and/or flax.

The nucleic acid sequence used is most preferably the sequence indicated in SEQ ID NO: 64.

It will be appreciated that the invention also encompasses those codon-optimized DNA sequences which code for a polypeptide having the activity of a Δ5-elongase and whose amino acid sequence is modified in one or more positions by comparison with the wild-type sequence but which still has substantially the same activity as the wild-type protein.

The nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41, preferably having the sequence

depicted in SEQ ID NO: 1,b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 or 42, preferably in SEQ ID SEQ ID NO: 2,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated a) or b) above, in particular of the nucleic acid sequence indicated in SEQ ID NO: 1, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the sequence indicated in SEQ ID NO: 1, and

e) nucleic acid sequences which code for an amino acid sequence and which have at least one, for example 2, 3, 4, 5, 6, 7 or 8, preferably all of the amino acid pattern indicated in SEQ ID NO: 43, 44, 45, 46, 47, 48, 49 or 50.

Amino acid pattern means short amino acid sequences which preferably comprise less than 50, particularly preferably less than 40 and especially from 10 to 40 and even more preferably from 10 to 30 amino acids.

For the present invention, the identity is ascertained preferably over the full length of the nucleotide or amino acid sequences of the invention, for example for the nucleic acid sequence indicated in SEQ ID NO: 64 over the full length of 903 nucleotides.

The nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 171, 173, 175, 177, 179, 181 or 183, especially having the sequence depicted in SEQ ID NO: 171,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 172, 174, 176, 178, 180, 182 or 184, especially for the amino acid sequence indicated in SEQ ID NO: 172,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 1, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the sequence indicated in SEQ ID NO: 171, and

e) nucleic acid sequences which code for an amino acid sequence and which have at least one, for example 2, 3, 4, 5, 6, 7 or 8, preferably all of the amino acid pattern indicated in SEQ ID NO: 185, 186, 187, 188, 189, 190, 191 or 192.

The nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity is in particular likewise a codon-optimized sequence according to the present invention, preferably the nucleic acid sequence depicted in SEQ ID NO: 122.

The nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 51, 53 or 55, preferably having the sequence depicted in SEQ ID NO: 51,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 52, 54 or 56, preferably for the amino acid sequence indicated in SEQ ID NO: 52,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated in a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 51, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the nucleic acid indicated under SEQ ID NO: 51, and

e) nucleic acid sequences which code for an amino acid sequence which have at least one, for example 2, 3, 4, 5, 6 or 7, preferably all of the amino acid pattern indicated in SEQ ID NO: 57, 58, 59, 60, 61, 62 or 63.

Further suitable nucleic acid sequences can be found by the skilled worker from the literature or the well-known gene libraries such as, for example, www.ncbi.nlm.nih.gov.

In a further preferred embodiment of the process, additionally one or more nucleic acid sequences which code for a polypeptide having the activity of an ω-3-desaturase and/or of a Δ4-desaturase are introduced into the plant.

The nucleic acid sequence which codes for a polypeptide having an ω-3-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 193 or 195, preferably the sequence depicted in SEQ ID NO: 193,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 194,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequence indicated in SEQ ID NO: 193 or 195 under stringent conditions, and

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95%, and especially at least 96%, 97%, 98% or 99%, identical to the sequence indicated in SEQ ID NO: 193 or 195.

The ω-3-desaturase advantageously used in the process of the invention makes it possible to shift from the ω-6 biosynthetic pathway to the ω-3 biosynthetic pathway, leading to a shift from $C_{18:2}$ to $C_{18:3}$ fatty acids. It is further advantageous for the ω-3-desaturase to convert a wide range of phospholipids such as phosphatidylcholine (= PC), phosphatidylinositol (= PIS) or phosphatidylethanolamine (= PE). Finally, desaturation products can also be found in the neutral lipids (= NL), that is to say in the triglycerides.

The nucleic acid sequence which codes for a polypeptide having a Δ4-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, preferably having the sequence depicted in SEQ ID NO: 77,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 78, 80, 82, 84, 86, 88, 90, 92 or 94, preferably for the amino acid sequence indicated in SEQ ID NO: 78,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated in a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 77, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the sequence indicated in SEQ ID NO: 77, and

e) nucleic acid sequences which code for an amino acid sequence which have at least one, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14, preferably all of the amino acid pattern indicated in SEQ ID NO: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

The Δ4-desaturase which is advantageously used in the process of the invention catalyzes the introduction of a double bond into the fatty acid docosapentaenoic acid, leading to formation of docosahexaenoic acid.

It is advantageous for the described process of the invention additionally to introduce further nucleic acids which code for enzymes of fatty acid or lipid metabolism into the plants in addition to the nucleic acid sequences which code for polypeptides having a Δ6-desaturase activity, a Δ6-elongase activity, a Δ5-desaturase activity and a Δ5-elongase activity, and to the nucleic acid sequences which are introduced if appropriate and which code for a polypeptide having an ω-3-desaturase activity and/or a Δ4-desaturase activity.

It is possible in principle to use all genes of fatty acid or lipid metabolism in combination with the nucleic acid sequences used in the process of the invention; genes of fatty acid or lipid metabolism selected from the group of acyl-CoA dehydrogenase(s), acyl-ACP (acyl carrier protein) desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s),acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) are preferably used in combination with the Δ6-elongase, Δ6-desaturase, Δ5-desaturase and the Δ5-elongase, and if appropriate the ω3-desaturase and/or the Δ4-desaturase, it being possible to use individual genes or a plurality of genes in combination.

The nucleic acids used in the process of the invention are advantageously expressed in vegetative tissues (somatic tissue). Vegetative tissue means in the context of this invention a tissue which is propagated through mitotic divisions. Tissue of this type also arises through asexual reproduction (apomixis) and propagation. Propagation is the term used when the number of individuals increases in consecutive generations. These individuals arising through asexual propagation are very substantially identical to their parents. Examples of such tissues are leaf, flower, root, stalk, runners above or below ground (side shoots, stolons), rhizomes, buds, tubers such as root tubers or stem tubers, bulb, brood bodies, brood buds, bulbuls or turion. Such tissues may also arise through pseudo vivipary, true vivipary or vivipary caused by humans. However, seeds arising through agamospermy, as are typical of Asteraceae, Poaceae or Rosaceae, are also included among the vegetative tissues in which expression advantageously takes place. The nucleic acids used in the process of the invention are expressed to a small extent or not at all in generative tissue (germ line tissue). Examples of such tissues are tissues arising through sexual reproduction, i.e. meiotic cell divisions, such as, for example, seeds arising through sexual processes.

A small extent means that, compared with vegetative tissue, the expression measured at the RNA and/or protein level is less than 5%, advantageously less than 3%, particularly advantageously less than 2%, most preferably less than 1; 0.5; 0.25 or 0.125%.

The nucleic acid sequences are particularly preferably expressed in the leaves of the transgenic plants. This has the advantage that the LCPUFAs produced according to the invention can be taken in by animals and humans directly by consuming the leaves, and no previous processing of the plant material is necessary.

Expression of the nucleic acid sequences of the invention in the leaf can be achieved by using constitutive or leaf-specific promoters.

"Constitutive promoters" are promoters which make expression possible in a large number of, preferably in all, tissues over a substantial period during plant development, preferably throughout plant development. A promoter from a plant or from a plant virus is preferably used. The promoter of the CaMV (cauliflower mosaic virus) 35S transcript (Franck et al. (1980) Cell 21: 285-294), the 19S CaMV promoter (US 5,352,605), the actin promoter from rice (McElroy et al. (1990) Plant Cell 2: 163-171), the legumin B promoter (GenBank Acc. No. X03677), the agrobacterium nopaline synthase promoter, the TR dual promoter, the agrobacterium octopine synthase promoter, the ubiquitin promoter (Holtorf et al. (1995) Plant

Mol. Biol. 29: 637-649), the Smas promoter, the cinnamoyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits, the pEMU promoter (Last et al. (1991) Theor. Appl. Genet. 81: 581-588), the MAS promoter (Velten et al. (1984) EMBO J. 3(12): 2723-2730), the histone H3 promoter from corn (Lepetit et al. (1992) Mol. Gen. Genet. 231: 276-285), the promoter of the nitrilase 1 gene from arabidopsis (GenBank Acc. No. U38846, nucleotides 3862-5325) and the promoter of a proline-rich protein from wheat (WO 91/13991) and further promoters which mediate constitutive gene expression. The promoter of the CaMV 35S transcript is particularly preferred.

It is in principle possible to use all naturally occurring constitutive promoters with their regulatory sequences like those mentioned above for the novel process. However, it is likewise possible to use synthetic promoters in addition or alone.

"Leaf-specific promoters" are promoters which show a high activity in the leaf and no or only low activity in other tissues. "Low activity" means in the context of the invention that the activity in other tissues is less than 20%, preferably less than 10%, particularly preferably less than 5% and most preferably less than 3, 2 or 1% of the activity in the leaf. Examples of suitable leaf-specific promoters are the promoters of the small subunit of rubisco (Timko et al. (1985) Nature 318: 579-582) and of the chlorophyll a/b-binding protein (Simpson et al. (1985) EMBO J. 4: 2723-2729).

The skilled worker is aware of further leaf-specific promoters, or he can isolate further suitable promoters with known methods. Thus, the skilled worker is able to identify leaf-specific regulatory nucleic acid elements with the aid of conventional methods of molecular biology, e.g. hybridization experiments or DNA-protein binding studies. This entails for example in a first step isolating the total poly(A)$^+$ RNA from leaf tissue of the desired organism from which the regulatory sequences are to be isolated, and setting up a cDNA library. In a second step, cDNA clones which are based on poly(A)$^+$ RNA molecules from a non-leaf tissue are used to identify, by means of hybridization, those clones from the first library whose corresponding poly(A)$^+$ RNA molecules accumulate only in leaf tissue. Subsequently, these cDNAs identified in this way are used to isolate promoters which have leaf-specific regulatory elements. Further PCR-based methods for isolating suitable leaf-specific promoters are additionally available to the skilled worker.

It is, of course, also possible for the nucleic acid sequences of the present invention to be expressed in the seeds of the transgenic plants by using seed-specific promoters which are

active in the embryo and/or in the endosperm. Seed-specific promoters can in principle be isolated both from dicotyledonous and from monocotyledonous plants. Preferred promoters are listed hereinafter: USP (unknown seed protein) and vicilin (*Vicia faba*) (Bäumlein et al. (1991) Mol. Gen Genet. 225(3): 459-467), napin (oilseed rape) (US 5,608,152), conlinin (flax) (WO 02/102970), acyl-carrier protein (oilseed rape) (US 5,315,001 and WO 92/18634), oleosin (*Arabidopsis thaliana*) (WO 98/45461 and WO 93/20216), phaseolin (*Phaseolus vulgaris*) (US 5,504,200), Bce4 (WO 91/13980), legume B4 (LegB4 promoter) (Bäumlein et al. (1992) Plant J. 2(2): 233-239), Lpt2 and lpt1 (barley) (WO 95/15389 and WO 95/23230), seed-specific promoters from rice, corn and wheat (WO 99/16890), Amy32b, Amy 6-6 and aleurain (US 5,677,474), Bce4 (oilseed rape) (US 5,530,149), glycinin (soybean) (EP 571 741), phosphoenolpyruvate carboxylase (soybean) (JP 06/62870), ADR 12-2 (soybean) (WO 98/08962), isocitrate lyase (oilseed rape) (US 5,689,040) or α-amylase (barley) (EP 781 849).

In a particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of produced LCPUFAs, especially of the C22 fatty acids, in the seed oil is at least 5% by weight, advantageously at least 6, 7, 8, 9 or 10% by weight, preferably at least 11, 12, 13, 14 or 15% by weight, particularly preferably at least 16, 17, 18, 19 or 20% by weight, very particularly preferably at least 25, 30, 35 or 40% by weight, of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of C22 fatty acids in the seed oil is at least 8% by weight of the seed oil content.

In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes

place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of docosahexaenoic acid in the seed oil is at least 1% by weight, preferably at least 1.1, 1.2, 1.3, 1.4 or 1.5% by weight, particularly preferably at least 1.6, 1.7, 1.8 or 1.9% by weight, especially at least 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8 or 2.9% by weight, further preferably at least 3, 3.5 or 4% by weight of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of docosahexaenoic acid in the seed oil is at least 1.9% by weight of the seed oil content. It is known to the skilled worker in this connection that to produce docosahexaenoic acid additionally one or more nucleic acid sequences which codes for a polypeptide having the activity of a Δ4-desaturase activity are required. A nucleic acid sequence which codes for a polypeptide having the activity of a Δ4-desaturase activity is advantageously selected from the group consisting of nucleic acid sequences having the sequence depicted in SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, preferably having the sequence depicted in SEQ ID NO: 77.

In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of docosahexaenoic acid in the seed oil is at least 1% by weight, preferably at least 1.1, 1.2, 1.3, 1.4 or 1.5% by weight, particularly preferably at least 1.6, 1.7, 1.8 or 1.9% by weight, especially at least 2, 2.1, 2.2, 2.5, 2.6, 2.7, 2.8 or 2.9% by weight, further preferably at least 3, 3.5 or 4% by weight of the seed oil content. In this case, the content of the produced LCPUFAs, especially of the C22 fatty acids, in the seed oil is at least 5% by weight, advantageously at least 6, 7, 8, 9 or 10% by weight, preferably at least 11, 12, 13, 14 or 15% by weight, particularly preferably at least 16, 17, 18, 19 or 20% by weight, very particularly preferably at least 25, 30, 35 or 40% by weight of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of docosahexaenoic acid in the seed

oil is at least 1.9% by weight of the seed oil content, with the content of C22 fatty acids in the seed oil being at least 8% by weight of the seed oil content.

Plant gene expression can also be achieved via a chemically inducible promoter (see a review in Gatz (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that the gene expression takes place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

Promoters which respond to biotic or abiotic stress conditions are also suitable, for example the pathogen-induced PRP1 gene promoter (Ward et al., Plant. Mol. Biol. 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the chill-inducible potato alpha-amylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Other promoters which are also particularly suitable are those which bring about the plastid-specific expression, since plastids constitute the compartment in which precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters, such as the viral RNA polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the Arabidopsis clpP promoter, described in WO 99/46394.

It will be appreciated that the polyunsaturated fatty acids produced according to the invention can be produced not only in intact transgenic plants but also in plant cell cultures or in callous cultures.

The polyunsaturated fatty acids produced in the process are advantageously bound in phospholipids and/or triacylglycerides, but may also occur as free fatty acids or else bound in the form of other fatty acid esters in the organisms. They may in this connection be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different phospholipids such as phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and/or phosphatidylserine and/or triacylglycerides, monoacyl-glycerides and/or diacylglycerides. The LCPUFAs EPA, DPA and DHA produced in the process are advantageously present in phosphatidylcholine and/or phosphatidylethanolamine and/or in the triacylglycerides. The triacylglycerides may additionally also comprise further fatty acids such as short-chain fatty acids having 4 to 6 C atoms, medium-chain fatty acids

having 8 to 12 C atoms or long-chain fatty acids having 14 to 24 C atoms. They preferably comprise long-chain fatty acids, particularly preferably C_{20} or C_{22} fatty acids.

The term "glyceride" is understood as meaning glycerol esterified with one, two or three carboxyl radicals (mono-, di- or triglyceride). "Glyceride" is also understood as meaning a mixture of various glycerides. The glyceride is preferably a triglyceride. The glyceride or glyceride mixture can comprise further additions, for example free fatty acids, antioxidants, proteins, carbohydrates, vitamins and/or other substances.

A"glyceride" for the purposes of the process according to the invention is furthermore understood as meaning derivatives which are derived from glycerol. In addition to the above-described fatty acid glycerides, these also include glycerophospholipids and glyceroglycolipids. Preferred examples which may be mentioned here are the glycerophospholipids such as lecithin (phosphatidylcholine), cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylacylglycerophospholipids.

Phospholipids are to be understood as meaning, for the purposes of the invention, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and/or phosphatidylinositol.

The fatty acid esters with polyunsaturated C_{18}-, C_{20}- and/or C_{22}-fatty acid molecules can be isolated in the form of an oil or lipid, for example in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three or four, preferably four, five or six double bonds, from the useful plants which have been used for the preparation of the fatty acid esters; advantageously, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of the phosphatidyl ester, especially preferably in the form of the triacylglycerides, phosphatidylcholine and/or phosphatidylethanolamine. In addition to these esters, the polyunsaturated fatty acids are also present in the plants as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free

fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

The LCPUFAs produced in the process of the invention are produced with a content of at least 4% by weight, advantageously of at least 5, 6, 7, 8, 9 or 10% by weight, preferably of at least 11, 12, 13, 14 or 15% by weight, particularly preferably of at least 16, 17, 18, 19, or 20% by weight, very particularly preferably of at least 25, 30, 35 or 40% by weight based on the total fatty acids in the transgenic plant. The fatty acids EPA, DPA and/or DHA produced in the process of the invention are moreover present with a content of in each case at least 5% by weight, preferably of in each case at least 6, 7, 8 or 9% by weight, particularly preferably of in each case at least 10, 11 or 12% by weight, most preferably of in each case at least 13, 14, 15, 16, 17, 18, 19 or 20% by weight based on the total fatty acids in the transgenic plant.

The fatty acids are advantageously produced in bound form. It is possible with the aid of the nucleic acids used in the process of the invention for these unsaturated fatty acids to be put on the sn1, sn2 and/or sn3 position of the advantageously produced triacylglycerides. Advantageously, at least 11% of the triacylglycerides are doubly substituted (meaning on the sn1 and sn2 or sn2 and sn3 positions). Triply substituted triacylglycerides are also detectable. Since a plurality of reaction steps take place from the starting compounds linoleic acid (C18:2) and linolenic acid (C18:3), the final products of the process, such as, for example, arachidonic acid (ARA) or eicosapentaenoic acid (EPA), do not result as absolute pure products; traces or larger amounts of the precursors are always also present in the final product. If, for example, both linoleic acid and linolenic acid are present in the initial plant, the final products such as ARA or EPA and/or DPA and/or DHA are also present as mixtures. The precursors should advantageously amount to not more than 20% by weight, preferably not more than 15% by weight, particularly preferably not as 10% by weight, very particularly preferably not more than 5% by weight based on the amount of the respective final product. Advantageously, only ARA or EPA and/or DPA and/or DHA are produced in the process of the invention, bound or as free acids, as final products in a transgenic plant.

Fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise 6 to 15% palmitic acid, 1 to 6% stearic acid; 7-85% oleic acid; 0.5 to 8% vaccenic acid, 0.1 to 1% arachic acid, 7 to 25% saturated fatty acids, 8 to 85% monounsaturated fatty acids and 60 to 85% polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Preferably at least 0.1; 0.2; 0.3;

0.4; 0.5; 0.6; 0.7; 0.8; 0.9 or 1% arachidonic acid in the total fatty acid content, are present as advantageous polyunsaturated fatty acid in the fatty acid ester or fatty acid mixtures. The fatty acid esters or fatty acid mixtures produced by the process of the invention further advantageously comprise fatty acids selected from the group of fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enonic acid), malvalic acid (8,9-methyleneheptadec-8-enonic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienonic acid), vernonic acid (9,10-epoxyoctadec-12-enonic acid), taric acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynonic acid, pyrulic acid (t10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynonic acid, petroselenic acid (cis-6-octadecenonic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid, catalpic acid (9t11t13c-octadecatrienoic acid), eleosteric acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienonic acid). In general, the aforementioned fatty acids are advantageously present only in traces in the fatty acid esters or fatty acid mixtures produced by the process of the invention, meaning that their occurrence, based on the total fatty acid content, is less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, particularly preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very particularly preferably less than 4%, 3%, 2% or 1%. In a further preferred form of the invention the occurrence of these aforementioned fatty acids, based on the total fatty acids, is less than 0.9%; 0.8%; 0.7%; 0.6% or 0.5%, particularly preferably less than 0.4%; 0.3%; 0.2%; 0.1%. The fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise less than 0.1% based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, C23:6$^{\Delta 3,8,12,15,18,21}$).

It is possible through the nucleic acid sequences used in the process of the invention to achieve an increase in the yield of LCPUFAs in the transgenic plants of at least 50%, advantageously of at least 80%, particularly advantageously of at least 100%, very particularly advantageously of at least 150%, compared with the non-transgenic plants.

Chemically pure polyunsaturated fatty acids or fatty acid compositions can also be synthesized by the processes described above. To this end, the fatty acids or the fatty acid compositions are isolated from the plants in the known manner, for example via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

In principle, all dicotyledonous or monocotyledonous useful plants are suitable for the process of the invention. Useful plants mean plants which serve to produce foods for humans and animals, to produce other consumables, fibers and pharmaceuticals, such as cereals, e.g. corn, rice, wheat, barley, millet, oats, rye, buckwheat; such as tubers, e.g. potato, cassava, sweet potato, yams etc.; such as sugar plants e.g. sugarcane or sugarbeet; such as legumes, e.g. beans, peas, broad bean etc.: such as oil and fat crops, e.g. soybean, oilseed rape, sunflower, safflower, flax, camolina etc., to mention only a few. Advantageous plants are selected from the group of plant families consisting of the families of Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreacea, Elaeagnaceae, Ericceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae and Valerianaceae.

Examples which may be mentioned are the following plants: Anacardiaceae such as the genera *Pistacia*, *Mangifera*, *Anacardium*, for example the genus and species *Pistacia vera* [pistachio], *Mangifer indica* (mango) or *Anacardium occidentale* (cashew), Asteraceae such as the genera *Calendula*, *Carthamus*, *Centaurea*, *Cichorium*, *Cynara*, *Helianthus*, *Lactuca*, *Locusta*, *Tagetes*, *Valeriana*, e.g. the genus and species *Calendula officinalis* (common marigold), *Carthamus tinctorius* (safflower), *Centaurea cyanus* (cornflower), *Cichorium intybus* (chicory), *Cynara scolymus* (artichoke), *Helianthus annus* (sunflower), *Lactuca sativa*, *Lactuca crispa*, *Lactuca esculenta*, *Lactuca scariola* L. ssp. sativa, *Lactus scariola* L. var. integrata, *Lactuca scariola* L. var. integrifolia, *Lactuca sativa* subsp. romana, *Locusta communis*, Valeriana locusta (lettuce), Tagetes lucida, *Tagetes erecta* or *Tagetes tenuifolia*

(French marigold), Apiaceae such as the genus *Daucus*, e.g. the genus and species *Daucus carota* (carrot), Betulaceae such as the genus *Corylus*, e.g. the genera and species *Corylus avellana* or *Corylus colurna* (hazelnut), Boraginaceae such as the genus *Borago*, e.g. the genus and species *Borago officinalis* (borage), Brassicaceae such as the genera *Brassica*, *Camelina*, *Melanosinapis*, *Sinapis*, *Arabadopsis*, e.g. the genera and species *Brassica napus*, *Brassica rapa* ssp. (oilseed rape), *Sinapis arvensis*, *Brassica juncea*, *Brassica juncea* var. juncea, *Brassica juncea* var. crispifolia, *Brassica juncea* var. foliosa, *Brassica nigra*, *Brassica sinapioides*, *Camelina sativa*, *Melanosinapis communis* (mustard), *Brassica oleracea* (feed beet) or *Arabidopsis thaliana*, Bromeliaceae such as the genera *Anana*, *Bromelia* (pineapple), e.g. the genera and species *Anana comosus*, *Ananas ananas* or *Bromelia comosa* (pineapple), Caricaceae such as the genus *Carica* such as the genus and species *Carica papaya* (papaya), Cannabaceae such as the genus *Cannabis* such as the genus and species *Cannabis sative* (hemp), Convolvulaceae such as the genera *Ipomoea*, *Convolvulus*, e.g. the genera and species *Ipomoea batatus*, *Ipomoea pandurata*, *Convolvulus batatas*, *Convolvulus tiliaceus, Ipomoea fastigiata*, *Ipomoea tiliacea*, *Ipomoea triloba* or *Convolvulus panduratus* (sweet potato, batate), Chenopodiaceae such as the genus *Beta* such as the genera and species *Beta vulgaris*, *Beta vulgaris* var. altissima, *Beta vulgaris* var. vulgaris, *Beta maritima*, *Beta vulgaris* var. perennis, *Beta vulgaris* var. conditiva or *Beta vulgaris* var. esculenta (sugarbeet), Cucurbitaceae such as the genus *Cucubita*, e.g. the genera and species *Cucurbita maxima*, *Cucurbita mixta*, *Cucurbita pepo* or *Cucurbita moschata* (pumpkin), Elaeagnaceae such as the genus *Elaeagnus*, e.g. the genus and species *Olea europaea* (olive), Ericaceae such as the genus *Kalmia*, e.g. the genera and species *Kalmia latifolia*, *Kalmia angustifolia*, *Kalmia microphylla*, *Kalmia polifolia*, *Kalmia occidentalis*, *Cistus chamaerhodendros* or *Kalmia lucida* (mountain laurel), Euphorbiaceae such as the genera *Manihot*, *Janipha*, *Jatropha*, *Ricinus*, e.g. the genera and species *Manihot utilissima*, *Janipha manihot*, *Jatropha manihot*, *Manihot aipil*, *Manihot dulcis*, *Manihot manihot*, *Manihot melanobasis*, *Manihot esculenta* (cassava) or *Ricinis communis* (castor oil plant), Fabaceae such as the genera *Pisum*, *Albizia*, *Cathormion*, *Feuillea*, *Inga*, *Pithecolobium*, *Acacia*, *Mimosa*, *Medicajo*, *Glycine*, *Dolichos*, *Phaseolus*, *Soja*, e.g. the genera and species *Pisum sativum*, *Pisum arvense, Pisum humile* (pea), *Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia*

julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa (acacia), *Medicago sativa, Medicago falcata, Medicago varia* (alfalfa) Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max (soybean), Geraniaceae such as the genera *Pelargonium*, *Cocos*, *Oleum*, e.g. the genera and species *Cocos nucifera, Pelargonium grossularioides or Oleum cocois* (coconut), Gramineae such as the genus *Saccharum*, e.g. the genus and species *Saccharum officinarum*, Juglandaceae such as the genera *Juglans*, *Wallia*, e.g. the genera and species *Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra* or *Wallia nigra* (walnut), Lauraceae such as the genera *Persea*, *Laurus*, e.g. the genera and species *Laurus nobilis* (bay), *Persea americana, Persea gratissima* or *Persea persea* (avocado), Leguminosae such as the genus *Arachis*. e.g. the genus and species *Arachis hypogaea* (peanut), Linaceae such as the genera *Linum*, *Adenolinum*, e.g. the genera and species *Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne* var. lewisii, *Linum pratense* or *Linum trigynum* (flax), Lythrarieae such as the genus *Punica*, e.g. the genus and species *Punica granatum* (pomegranate), Malvaceae such as the genus *Gossypium*, e.g. the genera and species *Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum* or *Gossypium thurberi* (cotton), Musaceae such as the genus *Musa*, e.g. the genera and species *Musa nana, Musa acuminata, Musa paradisiaca, Musa* spp. (banana), Onagraceae such as the genera *Camissonia*, *Oenothera*, e.g. the genera and species *Oenothera biennis* or *Camissonia brevipes* (evening primrose), Palmae such as the genus *Elaeis*, e.g. the genus and species *Elaeis guineensis* (oil palm), Papaveraceae such as the genus *Papaver*, e.g. the genera and species *Papaver orientale, Papaver rhoeas, Papaver dubium* (poppy), Pedaliaceae such as the genus *Sesamum* e.g the genus and species *Sesamum indicum* (sesame), Piperaceae such as the genera *Piper*, *Artanthe*, *Peperomia*, *Steffensia*, e.g. the genera and species *Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata* (cayenne pepper), Poaceae such as the genera *Hordeum, Secale, Avena, Sorghum,*

Andropogon, Holcus, Panicum, Oryza, Zea (corn), Triticum, e.g. the genera and species *Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum* (barley), *Secale cereale* (rye), *Avena sativa, Avena fatua, Avena byzantina, Avena fatua* var. sativa, *Avena hybrida* (oats), *Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum* (millet), *Oryza sativa, Oryza latifolia* (rice), *Zea mays* (corn), *Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum* or *Triticum vulgare* (wheat), Porphyridiaceae such as the genera *Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia*, e.g. the genus and species *Porphyridium cruentum*, Proteaceae such as the genus *Macadamia*, e.g. the genus and species *Macadamia intergrifolia* (macadamia), Rubiaceae such as the genus *Coffea*, e.g. the genera and species *Coffea* spp., *Coffea arabica, Coffea canephora* or *Coffea liberica* (coffee), Scrophulariaceae such as the genus *Verbascum*, e.g. the genera and species *Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus* (mullein), Solanaceae such as the genera *Capsicum, Nicotiana, Solanum, Lycopersicon*, e.g. the genera and species *Capsicum annuum, Capsicum annuum* var. glabriusculum, *Capsicum frutescens* (pepper), *Capsicum annuum* (paprika), *Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris* (tobacco), *Solanum tuberosum* (potato), *Solanum melongena* (aubergine), *Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium* or *Solanum lycopersicum* (tomato), Sterculiaceae such as the genus *Theobroma*, e.g. the genus and species *Theobroma cacao* (cocoa), or Theaceae such as the genus *Camellia*, e.g. the genus and species *Camellia sinensis* (tea).

In an advantageous embodiment of the process, the useful plants used are oil fruit plants which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower (*Carthamus tinctoria*), poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, verbascum, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, flax, soybean, pistachios, borage, trees (oil palm, coconut or walnut) or arable crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, egg plant and tomato, *Vicia* species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Advantageous plants according to the invention are oil fruit plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, flax, soybean, borage, trees (oilpalm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as sunflower, safflower, tobacco, verbascum, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, flax, hemp or thistle. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, flax, or hemp.

It is also advantageous to express the nucleic acid sequences of the invention in the leaves of feed or food plants and thus to increase the content of eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in the leaves. Preferred feed plants are, for example, trefoil species such as red clover (*Trifolium pratense*), white clover (*Trifolium repens*), alsike clover (*Trifolium hybridum*), sainfoin (*Onobrychis viccifolia*), Egyptian clover (*Trifolium alexandrinium*) and Persian clover (*Trifolium resupinatum*). Preferred food plants are for instance lettuce species such as *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola* L. ssp. sativa, *Lactuca scariola* L. var. integrata, *Lactuca scariola* L. var. integrifolia, *Lactuca sativa* subsp. romana, *Locusta communis* and *Valeriana locusta*.

It is possible through the enzymatic activity of the nucleic acid sequences which are used in the process of the invention and which code for polypeptides having Δ6-elongase, Δ6-desaturase, Δ5-desaturase and/or Δ5-elongase activity, advantageously in combination with nucleic acid sequences which code for polypeptides having ω3-desaturase and/or Δ4-desaturase activity, and further nucleic acid sequences which code for polypeptides of fatty acid or lipid metabolism, such as further polypeptides having Δ5-, Δ6-, Δ8-, Δ12-desaturase or Δ5-, Δ6- or Δ9-elongase activity, to produce a wide variety of polyunsaturated fatty acids

in the process of the invention. Depending on the useful plants chosen for use in the process of the invention, mixtures of the various polyunsaturated fatty acids or individual polyunsaturated fatty acids such as EPA, DPA or DHA can be produced in free or bound form. Depending on the prevailing fatty acid composition in the starting plant (C18:2 or C18:3 fatty acids), the resulting fatty acids are derived from C18:2 fatty acids, such as GLA, DGLA or ARA or are derived from C18:3 fatty acids, such as EPA, DPA or DHA. If the only unsaturated fatty acid present in the plant used for the process is linoleic acid (LA, $C18:2^{\Delta 9,12}$), the only possible products of the process are GLA, DGLA and ARA, which may be present as free fatty acids or bound. If the only unsaturated fatty acid present in the plant used in the process is α-linolenic acid (ALA, $C18:3^{\Delta 9,12,15}$), for example as in flax, the only possible products of the process are SDA, ETA, EPA, DPA and/or DHA, which may be present as described above as free fatty acids or bound. It is possible to produce in a targeted manner only individual products in the plant by modifying the activity of the enzymes used in the process and involved in the synthesis Δ6-elongase, Δ6-desaturase, Δ5-desaturase and/or Δ6-elongase, advantageously in combination with further genes of lipid or fatty acid metabolism. Advantageously, only EPA, DPA or DHA or mixtures thereof are synthesized. Since the fatty acids are synthesized in biosynthesis chains, the respective final products are not present as pure substances in the organisms. Small amounts of the precursor compounds are always also present in the final product. These small amounts are less than 20% by weight, advantageously less than 15% by weight, particularly advantageously less than 10% by weight, very particularly advantageously less than 5, 4, 3, 2 or 1% by weight based on the final products EPA, DPA or DHA or mixtures thereof.

To increase the yield in the process according to the invention for the production of oils and/or triglycerides with a polyunsaturated fatty acid, content which is advantageously increased, it is advantageous to increase the amount of starting product for the synthesis of fatty acids. This can be achieved for example by introducing a nucleic acid which encodes a polypeptide with Δ12-desaturase into the organism. This is particularly advantageous in useful plants, such as oil-producing plants such as plants of the Brassicaceae family, such as the genus *Brassica*, for example rape; the Elaeagnaceae family, such as the genus *Elaeagnus*, for example the genus and species *Olea europaea* or the family Fabaceae, such as the genus *Glycine*, for example the genus and species *Glycine max*, which are high in oleic acid. Since these organisms have an only low linoleic acid content (Mikoklajczak et al. (1961) Journal of the American Oil Chemical Society 38: 678 - 681) it is advantageous to use said Δ12-

desaturases for producing the starting material linolenic acid from oleic acid. It is also possible in addition for the starting fatty acids to be provided from outside, but this is less preferred for reasons of cost.

Mosses and algae are the only plant systems known to produce considerable amounts of polyunsaturated fatty acids such as arachidonic acid (ARA) and/or eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Mosses comprise PUFAs in membrane lipids, whereas algae, organisms related to algae, and some fungi also accumulate substantial amounts of PUFAs in the triacylglycerol fraction. Nucleic acid molecules isolated from strains which accumulate PUFAs also in the triacylglycerol fraction are therefore particularly advantageous for the process of the invention and thus for modifying the lipid and PUFA production system in a plant such as a useful plant such as an oil crop plant, for example oilseed rape, canola, flax, hemp, soybean, sunflower, borage. They can therefore advantageously be used in the process of the invention.

Nucleic acids used in the process of the invention are advantageously derived from plants such as algae, for example algae of the family of Prasinophyceae such as from the genera *Heteromastix, Mammella, Mantoniella, Micromonas, Nephroselmis, Ostreococcus, Prasinocladus, Prasinococcus, Pseudoscourfielda, Pycnoocus, Pyramimonas, Scherffelia* or *Tetraselmis* such as the genera and species *Heteromastix longifillis, Mamiella gilva, Mantoiella squamata, Micromonas pusilla, Nephroselmis olivacea, Nephroselmis pyriformis, Neproselmis rotunda, Ostreococcus tauri, Ostreococcus* sp. *Prasinocladus ascus, Prasinocladus lubricus, Pycnococcus provasolii, Pyramimonas amylifera, Pyramimonas disomata, Pyramimonas obovata, Pyramimonas orientalis, Pyramimonas parkae, Pyramimonas spinefera, Pyramimonas* sp., *Tetraselmis apiculta, Tetraselmis carteriaformis, Tetraselmis chui, Tetraselmis convolutae, Tetraselmis desikacharyi, Tetraselmis gracilis, Tetraselmis hazeni, Tetraselmis impellucida, Tetraselmis inconspicua, Tetraselmis levis, Tetraselmis maculata, Tetraselmis marina, Tetraselmis striata, Tetraselmis subcordiformis, Tetraselmis suecica, Tetraselmis tetrabrachia, Tetraselmis tetrathele, Tetraselmis verrucosa, Tetraselmis verrucosa* fo. rubens or *Tetraselmis* sp. or algae from the family Euglenacease such as from the genera *Ascoglena, Astasia, Colacium, Cyclidiopsis, Euglena, Euglenopsis, Hyalophacus, Khawkinea, Lepocinclis, Phacus, Strombomonas* or *Trachelomonas* such as the genera and species E*uglena acus, Euglena geniculata, Euglena gracilis, Euglena*

mixocylindracea, Euglena rostrifera, Euglena viridis, Colacium stentorium, Trachelomonas cylindrica or *Trachelomonas volvocina*.

Further advantageous plants are algae such as *Isochrysis* or *Crypthecodinium*, algae/diatoms such as *Thalassiosira* or *Phaeodactylum*, mosses such as *Physcomitrella* or *Ceratodon* or higher plants such as the Primulaceae such as *Aleuritia, Calendula stella, Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi such as *Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor* or *Mortierella*, bacteria such as shewanella, yeasts or animals such as nematodes such as *Caenorhabditis*, insects, frogs, sea cucumbers or fishes. The nucleic acid sequences isolated according to the invention are advantageously derived from an animal from the order of vertebrates. The nucleic acid sequences are preferably derived from the class of Vertebrata; Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus or Vertebrata, Amphibia, Anura, Pipidae, Xenopus or Evertebrata such as Protochordata, Tunicata, Holothuroidea, Cionidae such as *Amaroucium constellatum, Botryllus schlosseri, Ciona intestinalis, Molgula citrina, Molgula manhattensis, Perophora viridis* or *Styela partita*. The nucleic acids are particularly advantageously derived from fungi, animals or from plants such as algae or mosses, preferably from the order of Salmoniformes such as of the family of Salmonidae such as of the genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss, Trutta trutta* or *Salmo trutta* fario, from algae such as the genera *Mantoniella* or *Ostreococcus* or from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum* or from algae such as *Crypthecodinium*.

In a preferred embodiment, the process further comprises the step of obtaining a cell or a whole plant which comprises the nucleic acid sequences which are used in the process and which code for a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase and, if appropriate, nucleic acid sequences which code for an ω3-desaturase and/or a Δ4-desaturase, it being possible for the cell and/or the useful plant also to comprise further nucleic acid sequences of lipid or fatty acid metabolism. The nucleic acid sequences preferably used in the process are for expression advantageously incorporated into at least one gene construct and/or a vector as described hereinafter, alone or in combination with further nucleic acid sequences which code for proteins of fatty acid or lipid metabolism, and finally transformed into the cell or plant. In a further preferred embodiment, this process further comprises the step of obtaining the oils, lipids or free fatty acids from the useful plants, The cell produced in this

way or the useful plant produced in this way is advantageously a cell of an oil-producing plant, vegetable plant, lettuce plant, or ornamental plant or the plant itself as stated above.

Growing means for the cultivation in the case of plant cells, tissue or organs on or in a nutrient medium or of the whole plant on or in a substrate, for example in hydroculture, flower pot soil or on an arable field.

For the purposes of the invention, "transgenic" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (= gene construct) or a vector comprising the nucleic acid sequences used in the process according to the invention or a plant transformed with the nucleic acid sequences, expression cassette or vector used in the process according to the invention, all those constructions brought about by recombinant methods in which either

a) the nucleic acid sequence, or

b) a genetic control sequence which is operably linked with the nucleic acid sequence, for example a promoter, or

c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to be, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment means the natural genomic or chromosomal locus in the original organism or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, very especially preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequence used in the process according to the invention with the nucleic acid sequence which encodes proteins with corresponding Δ6-desaturase, Δ6-elongase, Δ5-desaturase and Δ5-elongase activity, advantageously in combination with nucleic acid sequences which encode proteins having ω3-desaturase and/or Δ4-desaturase activity – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic

("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

A “transgenic plant” for the purposes of the invention is understood as mentined above as meaning that the nucleic acids used in the process are not at their natural locus in the genome of the plant. In this case, it is possible for the nucleic acid sequences to be expressed homologously or heterologously. However, transgenic also means that, while the nucleic acids according to the invention are at their natural position in the genome of the plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids used in the process according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acid sequences takes place.

Preferred transgenic organisms are useful plants such as oil-producing plants, vegetable plants, lettuce plants or ornamental plants which are advantageously selected from the group of plant families consisting of the families of Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae and Valerianaceae.

Host plants which are suitable for the nucleic acids, the expression cassette or the vector used in the process according to the invention are, in principle, advantageously all useful plants which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and which are suitable for the expression of recombinant genes. Examples which should be mentioned at this point are plants such as *Arabidopsis*, Asteraceae such as *Calendula* or useful plants such as soybean, peanut, castor-oil plant, sunflower, maize, cotton, flax, oilseed rape, coconut, oil palm, safflower (*Carthamus tinctorius*) or cacao bean. Further advantageous plants are mentioned at other points in this application.

Microorganisms are generally used as intermediate hosts for the production of transgenic useful plants. Such utilizable intermediate host cells are detailed in: Goeddel, Gene

Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990).

Expression strains which can advantageously be used for this purpose are, for example, those with a lower protease activity. They are described, for example, in: Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128.

Transgenic plants which comprise the polyunsaturated, long-chain fatty acids synthesized in the process according to the invention can advantageously be marketed directly without there being any need for the oils, lipids or fatty acids synthesized to be isolated. This form of marketing is particularly advantageous.

"Plants" for the purposes of the present invention are intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue.

The compounds produced in the process of the invention can, however, also be isolated from the plants in the form of their oils, fat, lipids and/or free fatty acids. Polyunsaturated fatty acids produced by the process of the invention can be obtained by harvesting the plants or plant cells either from the culture in which they grow or from the field. This can take place by pressing or extracting the plant parts, preferably the plant seeds. It is possible in this connection for the oils, fats, lipids and/or free fatty acids to be obtained by pressing by so-called cold drawing or cold pressing without input of heat. To make it easier to break open the plant parts, specifically the seeds, they are previously crushed, steamed or roasted. The seeds pretreated in this way can then be pressed or extracted with solvent such as warm hexane. The solvent is then removed again. It is possible in this way to isolate more than 96% of the compounds produced in the process of the invention. The products obtained in this way are then processed further, that is to say refined. This entails initially for example the plant mucilage and suspended matter being removed. So-called desliming can take place enzymatically or, for example, chemically/physically by adding acid such as phosphoric acid. The free fatty acids are then removed by treatment with a base, for example sodium hydroxide solution. The resulting product is thoroughly washed with water to remove the

alkali remaining in the product, and is dried. In order to remove the coloring matters still present in the product, the products are subjected to a bleaching with, for example, bleaching earth or activated carbon. Finally, the product is also deodorized for example with steam.

The PUFAs or LCPUFAs produced by this process are preferably C_{20} and/or C_{22} fatty acid molecules having at least four double bonds in the fatty acid molecule, preferably five or six double bonds. These C_{20} and/or C_{22} fatty acid molecules can be isolated from the plant in the form of an oil, lipid or a free fatty acid. Suitable transgenic plants are for example those mentioned above.

These oils, lipids or fatty acids of the invention comprise, as described above, advantageously 6 to 15% palmitic acid, 1 to 6% stearic acid; 7 - 85% oleic acid; 0.5 to 8% vaccenic acid, 0.1 to 1% arachic acid, 7 to 25% saturated fatty acids, 8 to 85% monounsaturated fatty acids and 60 to 85% polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the plants.

Advantageous polyunsaturated, long-chain fatty acids present in the fatty acid esters or fatty acid mixtures such as phosphatidyl fatty acid esters or triacylglyceride esters are preferably at least 10; 11; 12; 13; 14; 15; 16; 17; 18; 19 or 20% by weight based on the total fatty acid content of eicosapentaenoic acid, based on the total fatty acid content, and/or at least 1; 2; 3; 4; 5 or 6% by weight of docosapentaenoic acid, based on the total fatty acid content, and/or at least 1; 2; 3; preferably at least 4; 5; 6; particularly preferably at least 7 or 8 and most preferably at least 9 or 10% by weight of docosahexaenoic acid, based on the total fatty acid content.

The fatty acid esters or fatty acid mixtures which have been produced by the process of the invention further comprise fatty acids selected from the group of fatty acids erucic acid (13-docosaic acid), sterculic acid (9,10-methylene octadec-9-enonic acid), malvalic acid (8,9-methylene heptadec-8-enonic acid), chaulmoogrinic acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienonoic acid), vernonoic acid (9,10-epoxyoctadec-12-enonoic acid), tarinic acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynonic acid, pyrulic acid (t10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynonic acid, petroselenic acid (cis-6-octadecenonic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid, catalpic acid (9t11t13c-octadecatrienoic acid), eleosteric acid (9c11t13t-

octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienonic acid). In general, the aforementioned fatty acids are advantageously present only in traces in the fatty acid esters or fatty acid mixtures produced by the process of the invention, meaning that their occurrence, based on the total fatty acid content, is less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, particularly preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very particularly preferably less than 4%, 3%, 2% or 1%. In a further preferred form of the invention the occurrence of these aforementioned fatty acids, based on the total fatty acids, is less than 0.9%; 0.8%; 0.7%; 0.6% or 0.5%, particularly preferably less than 0.4%; 0.3%; 0.2%; 0.1%. The fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise less than 0.1% based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, C23:6$^{\Delta 3,8,12,15,18,21}$).

A further embodiment according to the invention is the use of the oils, the lipids, the fatty acids and/or the fatty acid composition, which are produced by the process of the invention, in feeding stuffs, foodstuffs, cosmetics or pharmaceuticals. The oils, lipids, fatty acids or fatty acid mixtures obtained in the process according to the invention can be used for admixture with other oils, lipids, fatty acids or fatty acid mixtures of animal origin, such as, for example, fish oils, in the manner with which the skilled worker is familiar. These oils, lipids, fatty acids or fatty acid mixtures which are produced in this way and consist of vegetable and animal components can also be used for the preparation of feeding stuffs, foodstuffs, cosmetics or pharmaceuticals.

The term “oil”, “lipid“ or “fat” is understood as meaning a fatty acid mixture comprising unsaturated and/or saturated, preferably esterified fatty acid(s). It is preferred that the oil, fat or lipid is high in polyunsaturated free or advantageously esterified fatty acid(s), in particular linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid. Preferably, the amount of unsaturated esterified fatty acids is approximately 30%, with an amount of 50% being especially preferred and an amount of 60%, 70%, 80% or more being most preferred. The amount of the fatty acid can be

determined by gas chromatography after converting the fatty acids into the methyl esters by transesterification. The oil, lipid or fat can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and the like. In particular, the amount of the various fatty acids can vary, depending on the starting plant.

As described above, the polyunsaturated fatty acid esters advantageously having three, four, five or six, particularly advantageously having five or six double bonds and which have been prepared in the process advantageously take the form of fatty acid esters, for example, sphingolipid esters, phosphoglyceride esters, lipid esters, glycolipid esters, phospholipid esters, monoacylglycerol esters, diacylglycerol esters, triacylglycerol esters or other fatty acid esters, preference being given to phospholipid esters and/or triacylglycerol esters.

Starting with the polyunsaturated fatty acid esters produced thus in the process according to the invention and advantageously having at least three, four, five or six double bonds, the polyunsaturated fatty acids which are present can be liberated for example via treatment with alkali, for example with aqueous KOH or NaOH, or by acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification with, for example, H_2SO_4. However, the fatty acids can also be liberated directly without the above-described processing steps.

Substrates of the nucleic acid sequences used in the process which encode polypeptides with Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase activity and optionally nucleic acid sequences which encode polypetides having ω3-desaturase and/or Δ4-desaturase activity, and/or of the further nucleic acids which are used, such as the nucleic acid sequences which encode polypeptides of the fatty acid or lipid metabolism selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously C_{16}-, C_{18}- or C_{20}-fatty acids. Preferably, the fatty acids converted in the process as substrates are converted in the form of their acyl-CoA esters and/or in the form of their phospholipid esters.

To produce the long-chain PUFAs according to the invention, the saturated, monounsaturated C_{16}-fatty acids and/or polyunsaturated C_{18}-fatty acids must first, depending on the substrate, be desaturated and/or elongated or only deaturated by the enzymatic activity of a desaturase and/or elongase and subsequently elongated by at least two carbon atoms by an elongase. After one elongation cycle, this enzyme activity leads either starting from C_{16}-fatty acids to C_{18}-fatty acids or starting from C_{18}-fatty acids to C_{20}-fatty acids, and after two elongation cycles starting from C_{16}-fatty acids leads to C_{20}-fatty acids. The activity of the desaturases or elongases used in the process according to the invention preferably leads to C_{20}- and/or C_{22}-fatty acids, advantageously with at least two or three double bonds in the fatty acid molecule, preferably with four, five or six double bonds, especially preferably to C_{22}-fatty acids with at least five double bonds in the fatty acid molecule. Especially preferred products of the process according to the invention are eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid. The C_{18}-fatty acids with at least two double bonds in the fatty acid can be elongated by the enzymatic activity according to the invention in the form of the free fatty acid or in the form of the esters, such as phospholipids, glycolipids, sphingolipids, phosphoglycerides, monoacylglycerol, diacylglycerol or triacylglycerol.

The preferred biosynthesis site of fatty acids, oils, lipids or fats in the plants which are advantageously used is, for example, generally the seed or cell layers of the seed, so that seed-specific expression of the nucleic acids used in the process makes sense. However, it is obvious that the biosynthesis of fatty acids, oils or lipids need not be limited to the seed tissue, but may also take place in a tissue specific manner in all of the remaining parts of the plant, for example in epidermal cells or in the tubers. The synthesis advantageously takes place according to the inventive process in the vegetative (somatic) tissue.

Owing to the method according to the invention, the polyunsaturated fatty acids which are produced can, in principle, be increased in two ways in the plants used in the process. Advantageously the pool of free polyunsaturated fatty acids and/or the amount of the esterified polyunsaturated fatty acids produced by the process can be increased. Advantageously, the pool of esterified polyunsaturated fatty acids in the transgenic plants is increased by the process according to the invention, advantageously in the form of the phosphatidyl esters and/or triacyl esters.

The sequences used in the process of the invention are cloned singly into expression constructs or provided on a joint recombinant nucleic acid molecule and used for introduction

and for expression in organisms. These expression constructs make it possible for the polyunsaturated fatty acids produced in the process of the invention to be synthesized optimally.

The nucleic acids used in the process may, after introduction into a plant or plant cell, either be located on a separate plasmid or advantageously be integrated into the genome of the host cell. In the case of integration into the genome, the integration may be random or take place by recombination such that the native gene is replaced by the introduced copy, thus modulating production of the desired compound by the cell, or through use of a gene in trans, so that the gene is functionally connected to a functional expression unit which comprises at least one sequence ensuring the expression of a gene and at least one sequence ensuring the polyadenylation of a functionally transcribed gene. The nucleic acid sequences are advantageously introduced into the plants via multiexpression cassettes or constructs for multiparallel expression, i.e. the nucleic acid sequences are present in a joint expression unit.

The nucleic acid construct may comprise more than one nucleic acid sequence coding for a polypeptide having the enzymatic activity of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase, and/or ω3-desaturase. It is also possible for a plurality of copies of a nucleic acid sequence coding for a polypeptide having the enzymatic activity of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase, and/or ω3-desaturase to be present.

For the introduction, the nucleic acids used in the process are advantageously amplified and ligated in the known manner. Preferably, a procedure following the protocol for Pfu DNA polymerase or a Pfu/Taq DNA polymerase mixture is followed. The primers are selected depending on the sequence to be amplified. The primers should expediently be chosen in such a way that the amplicon comprises the entire codogenic sequence from the start codon to the stop codon. After the amplification, the amplificon is expediently analyzed. For example, the analysis can be carried out by gel-electrophoretic separation with respect to quality and quantity. Thereafter, the amplicon can be purified following a standard protol (for example Qiagen). An aliquot of the purified amplicon is then available for the subsequent cloning step. Suitable cloning vectors are generally known to the skilled worker. These include, in particular, vectors which are capable of replication in microbial systems, that is to say mainly vectors which ensure efficient cloning in yeasts or fungi and which make possible the stable transformation of plants. Those which must be mentioned in particular are various binary and

cointegrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they comprise at least the vir genes required for the Agrobacterium-mediated transformation and the T-DNA-delimiting sequences (T-DNA border). These vector systems preferably also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers, by means of which suitably transformed organisms can be identified. While in the case of cointegrated vector systems vir genes and T-DNA sequences are arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir genes. Owing to this fact, the last-mentioned vectors are relatively small, easy to manipulate and capable of replication both in E. coli and in Agrobacterium. These binary vectors include vectors from the series pBIB-HYG, pPZP, pBecks, pGreen. In accordance with the invention, pBin19, pBI101, pBinAR, pGPTV and pCAMBIA are used by preference. An overview of the binary vectors and their use is found in Hellens et al. (2000) Trends in Plant Science 5: 446–451.

In order to prepare the vectors, the vectors can first be linearized with restriction endonuclease(s) and then modified enzymatically in a suitable manner. Thereafter, the vector is purified, and an aliquot is employed for the cloning step. In the cloning step, the enzymatically cleaved and, if appropriate, purified amplificate is cloned with vector fragments which have been prepared in a similar manner, using ligase. In this context, a particular nucleic acid construct, or vector or plasmid construct, can have one or more than one codogenic gene segments. The codogenic gene segments in these constructs are preferably linked operably with regulatory sequences. The regulatory sequences include, in particular, plant sequences such as the above-described promoters and terminators. The constructs can advantageously be stably propagated in microorganisms, in particular Escherichia coli and Agrobacterium tumefaciens, under selective conditions and thus make possible the transfer of heterologous DNA into plants.

The nucleic acid sequences and nucleic acid constructs used in the inventive process can be introduced into microorganisms and then into plants, advantageously using cloning vectors, and thus be used in the transformation of plants such as those which are published in and cited therein: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, p. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic

Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. (1991) 42: 205-225. Thus, the nucleic acids, nucleic acid constructs and/or vectors used in the process can be used for the recombinant modification of a broad spectrum of plants so that the latter become better and/or more efficient LCPUFA producers.

Owing to the introduction of a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and Δ5-elongase gene into a plant, alone or in combination with other genes, it is not only possible to increase biosynthesis flux towards the end product, but also to increase, or to create *de novo* the corresponding triacylglycerol and/or phosphatidylester composition. Likewise, the number or activity of other genes which are involved in the import of nutrients which are required for the biosynthesis of one or more fatty acids, oils, polar and/or neutral lipids, can be increased, so that the concentration of these precursors, cofactors or intermediates within the cells or within the storage compartment is increased, whereby the ability of the cells to produce PUFAs, as described below, is enhanced further. By optimizing the activity or increasing the number of one or more of the Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase genes which are involved in the biosynthesis of these compounds, or by destroying the activity of one or more genes which are involved in the degradation of these compounds, it may be possible to increase the yield, production and/or production efficiency in fatty acid and lipid molecules from organisms and advantageously from plants.

The nucleic acid molecules used in the process of the invention code for proteins or parts thereof, whereas the proteins or the individual protein or parts thereof comprises an amino acid sequence which has sufficient homology to an amino acid sequence which is depicted in the sequences SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172 or SEQ ID NO: 52 and, if appropriate, SEQ ID NO: 194 or SEQ ID NO: 78, so that the proteins or parts thereof still have a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase activity and, if appropriate, a Δ4-desaturase and/or ω3-desaturase activity. The proteins or parts thereof which is/are encoded by the nucleic acid molecule/nucleic acid molecules preferably still have its/their essential enzymatic activity and the ability to participate in the metabolism of compounds necessary for constructing cell membranes or lipid bodies in organisms, advantageously in plants, or in the transport of molecules across these membranes. The proteins encoded by the nucleic acid molecules are at least about 60% and preferably at least

about 70%, 80% or 90%, and particularly preferably at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequences depicted in SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78. Homology or homologous means in the context of the invention identity or identical.

The homology was calculated over the entire amino acid or nucleic acid sequence region. To compare various sequences, the skilled worker has available a series of programs which are based on various algorithms. The algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution (1987) 25: 351-360; Higgins et al. (1989) CABIOS 5: 151–153) or the programs Gap and BestFit (Needleman and Wunsch (1970) J. Mol. Biol. 48: 443-453 and Smith and Waterman (1981) Adv. Appl. Math. 2: 482-489), which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used to carry out the sequence comparisons. The sequence homology data given above in % were determined over the entire sequence region using the program GAP with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for sequence comparisons.

Essential enzymatic activity of the ω3-desaturase, Δ6-desaturase, Δ6-elongase, Δ5-elongase, Δ4-desaturase and/or Δ5-desaturase used in the process of the invention means that, compared with the proteins/enzymes encoded by the sequence having SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, they still have an enzymatic activity of at least 10%, preferably of at least 20%, particularly preferably of at least 30% and most preferably of at least 40, 50 or 60%, and thus are able to participate in the metabolism of compounds necessary for synthesizing fatty acids, advantageously fatty acid esters such as phosphatidyl esters and/or triacylglyceride esters, in a plant or plant cell, or in the transport of molecules across membranes.

Nucleic acids which can be advantageously used in the process are derived from bacteria, fungi, diatoms, animals such as *Caernorhabditis* or *Oncorhynchus* or plants such as algae or mosses such as the genera *Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Pytium irregulare, Mantoniella, Ostreococcus, Isochrysis, Aleurita, muscarioides, Mortierella, Borago, Phaeodactylum, Crypthecodinium*, specifically

from the genera and species *Pytium irregulare, Oncorhynchus mykiss, Xenopus laevis, Ciona intestinalis, Thalassiosira pseudonona, Mantoniella squamata, Ostreococcus* sp., *Ostreococcus tauri, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium* sp., *Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricornutum, Caenorhabditis elegans* or particularly advantageously from *Pytium irregulare, Thraustochytrium* sp. and/or *Ostreococcus tauri*.

It is possible additionally to use in the process of the invention nucleotide sequences which code for a Δ12-desaturase, Δ9-elongase or Δ8-desaturase. The nucleic acid sequences used in the process are advantageously introduced in an expression cassette which makes expression of the nucleic acids in plants possible.

The nucleic acid sequences which code for the Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase are functionally linked to one or more regulatory signals to increase the gene expression. These regulatory sequences are intended to make targeted expression of the genes possible. This may mean for example, depending on the plant, that the gene is expressed and/or overexpressed only after induction, or that it is expressed and/or overexpressed immediately. Sequences advantageously used for the expression make constitutive expression possible, such as CaMV35S, CaMV36S, CaMV35Smas, nos, mas, ubi, stpt, lea or Super promoter. Expression preferably takes place in vegetative tissue as described above. In another preferred embodiment, the expression takes place in seeds.

These regulatory sequences are for example sequences to which inducers or repressors bind and thus regulate the expression of the nucleic acid. In addition to the regulatory sequences which are not linked in their natural locus to the nucleic acid sequences, or instead of these sequences, the natural regulation of these sequences may still be present before the actual structural genes and, if appropriate, have been genetically modified so that natural regulation is switched off and expression of the genes is increased. The gene construct may additionally advantageously also comprise one or more so-called “enhancer sequences” functionally linked to the promoter, which make increased expression of the nucleic acid sequence possible. Additional advantageous sequences can also be inserted at the 3’ end of the DNA sequences, such as further regulatory elements or terminators. Advantageous terminators are for example viral terminators such as the 35S terminator or others. The nucleic acid

sequences used in the process according to the invention may be present in one or more copies of the expression cassette (= gene construct). Preferably, only one copy of the genes is present in each expression cassette. This gene construct, or the gene constructs, can be introduced into the plant simultaneously or successively and expressed together in the host organism. In this context, the gene construct(s) can be inserted in one or more vectors and be present in the cell in free form, or else be inserted in the genome. It is advantageous for the insertion of further genes in the plant when the genes to be expressed are present together in one gene construct. However, it is also possible to introduce in each case one gene construct containing a nucleic acid sequence into a plant and to cross the resulting plants with one another in order to obtain progeny which contains all gene contructs together.

In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the gene expression of the genes introduced, thus enhancing it. Thus, an enhancement of the regulatory elements, advantageously at the transcriptional level, may take place by using strong transcription signals such as promoters and/or enhancers. In addition, however, enhanced translation is also possible, for example by improving the stability of the mRNA.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations, each of the nucleic acids which encode Δ6-desaturase, Δ6-elongase, Δ5-desaturase or Δ5-elongase and if appropriate the ω3-desaturase or Δ4-desaturase and which are used in the process should be expressed under the control of a separate promoter. This can be identical or different for each of the sequences. In this context, the expression cassette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site for insertion of the nucleic acid to be expressed, which cleavage site is advantageously in a polylinker. If appropriate, a terminator can be positioned behind the polylinker. This sequence is repeated several times, preferably three, four, five or six times, so that up to six genes can be combined in one construct and thus introduced into the transgenic plant in order to be expressed. To express the nucleic acid sequences, the latter are inserted behind the promoter via the suitable cleavage site, for example in the polylinker. Advantageously, each nucleic acid sequence has its own promoter and, if appropriate, its own terminator. However, it is also possible to insert a plurality of nucleic acid sequences behind a promoter and, if appropriate, before a terminator. Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to

say a nucleic acid sequence can be inserted at the first or last position in the cassette without the expression being substantially influenced by the position. In an advantageous embodiment, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminators can be used in the expression cassette. In a further advantageous embodiment, identical promoters such as the CaMV35S promoter can also be used.

As described above, the transcription of the genes which have been introduced should advantageously be terminated by suitable terminators at the 3' end of the biosynthesis genes which have been introduced (behind the stop codon). An example of a sequence which can be used in this context is the OCS 1 or the 35SCaMV terminator. As is the case with the promoters, different terminator sequences should be used here for each gene.

As described above, the gene construct can also comprise further genes to be introduced into the organisms. It is possible and advantageous to introduce into the host plants, and to express therein, regulatory genes such as genes for inductors, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a biosynthesis pathway. These genes can be of heterologous or of homologous origin. Moreover, further biosynthesis genes of the fatty acid or lipid metabolism can advantageously be present in the nucleic acid construct, or gene construct or alternatively, these genes can also be present on one further or more further nucleic acid constructs. A biosynthesis gene of the fatty acid or lipid metabolism which is preferably chosen is one or more genes selected from the group of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl- transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) or combinations thereof. Especially advantageous nucleic acid sequences are biosynthesis genes of the fatty acid or lipid metabolism selected from the group of the acyl-CoA:lysophospholipid acyltransferase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase and/or Δ9-elongase.

In this context, the abovementioned nucleic acids or genes can be cloned into expression cassettes, like those mentioned above, in combination with other elongases and desaturases and used for transforming plants with the aid of *Agrobacterium*.

The term "vector" used in this description relates to a nucleic acid molecule which is capable of transporting another nucleic acid to which it is bound. One type of vector is a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated. A further type of vector is a viral vector, it being possible for additional DNA segments to be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they have been introduced (for example bacterial vectors with bacterial replication origin). Other vectors are advantageously integrated into the genome of a host cell when they are introduced into the host cell, and thus replicate together with the host genome. Moreover, certain vectors can govern the expression of genes with which they are in operable linkage. These vectors are referred to in the present context as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids. In the present description, "plasmid" and "vector" can be used exchangeably since the plasmid is the form of vector which is most frequently used. However, the invention is also intended to cover other forms of expression vectors, such as viral vectors, which exert similar functions. Furthermore, the term vector is also intended to encompass other vectors with which the skilled worker is familiar, such as phages, viruses such as SV40, CMV, TMV, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA.

The recombinant expression vectors advantageously used in the process comprise the nucleic acid sequences or the above-described gene construct used in the process in a form which is suitable for expressing the nucleic acids used in a host cell, which means that the recombinant expression vectors comprise one or more regulatory sequences, which are selected on the basis of the host cells to be used for the expression, which regulatory sequence(s) is/are linked operably with the nucleic acid sequence to be expressed. In a recombinant expression vector, "linked operably" means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the expression of the nucleotide sequence is made possible and they are bound to each other in such a way that both sequences carry out the predicted function which is ascribed to the sequence (for example in an in-vitro transcription/translation system, or in a host cell if the vector is introduced into the host cell). The term "regulatory sequence" is intended to comprise promoters, enhancers and other expression control elements (for example polyadenylation signals). These regulatory sequences are described, for example, in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Florida,

Ed.: Glick and Thompson, Chapter 7, 89-108, including the references cited therein. Regulatory sequences comprise those which govern the constitutive expression of a nucleotide sequence in many types of host cell and those which govern the direct expression of the nucleotide sequence only in specific host cells under specific conditions. The skilled worker knows that the design of the expression vector can depend on factors such as the choice of host cell to be transformed, the desired degree of expression of the protein and the like.

The recombinant expression vectors used can be designed for the expression of the nucleic acid sequences used in the process in such a way that they can be transformed into prokaryotic intermediate hosts and finally, after introduction into the plants, make expression of the genes possible therein. This is advantageous because on account of simplicity, intermediate steps in vector construction are frequently carried out in microorganisms. For example, the Δ6-desaturates, Δ6-elongase, Δ5-desaturate and/or Δ5-elongase genes can be expressed in bacterial cells, insect cells (using baculovirus expression vectors), yeast cells and other fungal cells (see Romanos, M.A., et al. (1992) Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Editors, pp. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J., & Punt, P.J. (1992) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Editors, pp. 1-28, Cambridge University Press: Cambridge), Algae (Falciatore et al. (1999) Marine Biotechnology.1: (3):239-251), ciliates, with vectors following a transformation process as described in WO 98/01572, and preferably in cells of multicellular plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" Plant Cell Rep.:538-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter 6/7, pp. 71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer in: Transgenic Plants, vol. 1, Engineering and Utilization, Editors.: Kung and R. Wu, Academic Press (1993), 128-43; Potrykus (1991) Annu. Rev. Plant Physiol. Plant Molec. Biol. 42: 205-225 (and references cited therein)). Suitable hosts are what are further discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). The recombinant expression vector may alternatively be transcribed and translated in vitro for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes usually takes place with vectors which comprise constitutive or inducible promoters which control the expression of fusion or non-fusion proteins. Typical fusion expression vectors are inter alia pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), of which glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused to the recombinant target protein.

Examples of suitable inducible non-fusion E. coli expression vectors are inter alia pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression by the pTrc vector is based on transcription by host RNA polymerase from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector is based on transcription from a T7-gn10-lac fusion promoter which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ prophage which harbors a T7 gn1 gene under transcription control of the lacUV 5 promoter.

Other vectors suitable in prokaryotic organisms are known to the skilled worker; these vectors are for example in E. coli pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCI, in streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in bacillus pUB110, pC194 or pBD214, in corynebacterium pSA77 or pAJ667.

In a further embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in the yeast S. cerevisiae include pYeDesaturasec1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for constructing vectors suitable for use in other fungi, such as the filamentous fungi, are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., editors, pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi (J.W. Bennet & L.L. Lasure, Editors, pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are for

example pAG-1, YEp6, YEp13 or pEMBLYe23.

Alternatively, the nucleic acid sequences used in the process of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expressing proteins in cultured insect cells (e.g. Sf9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The above mentioned vectors provide only a small survey of possible suitable vectors. Further plasmids are known to the skilled worker and are described for example in: Cloning Vectors (Editors Pouwels, P.H. et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Further suitable expression systems for prokaryotic and eukaryotic cells see in chapters 16 and 17 of Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

The genes used in the process can also be expressed in single-celled plant cells (such as algae), see Falciatore et al. (1999) Marine Biotechnology 1 (3):239-251 and references cited therein, and in plant cells from higher plants (for example spermatophytes such as arable crops). Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) Plant Mol. Biol. 20:1195-1197; and Bevan, M.W. (1984) Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38.

A plant expression cassette preferably comprises regulatory sequences which are capable of governing the expression of genes in plant cells and are linked operably so that each sequence can fulfil its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as gene 3 of the Ti plasmid pTiACH5 (Gielen et al., (1984) EMBO J. 3 835 et seq.), which is known as octopine synthase, or functional equivalents thereof, but all other terminators which are functionally active in plants are also suitable.

Since the regulation of plant gene expression is very often not limited to the transcriptional level, a plant expression cassette preferably comprises other sequences which are linked operably, such as translation enhancers, for example the overdrive sequence, which enhances

the tobacco mosaic virus 5' - untranslated leader sequence, which increases the protein/RNA ratio (Gallie et al. (1987) Nucl. Acids Research 15:8693-8711).

As described above, the plant gene expression must be linked operably with a suitable promoter which controls gene expression. Advantageously utilizable promoters are constitutive promoters (Benfey et al., EMBO J. (1989) 8: 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al. (1980) Cell 21: 285-294), 19S CaMV (see also US 5352605 and WO 84/02913), or plant promoters, such as the promoter of the Rubisco small subunit, which is described in US 4,962,028.

Other preferred sequences for use for functional connection in plant gene expression cassettes are targeting sequences which are necessary for guiding the gene product into its appropriate cellular compartment, for example into the vacuoles, the cell nucleus, all types of plastids such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells; (see a review in Kermode (1996) Crit. Rev. Plant Sci. 15(4): 284-423 and literature cited therein).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via traditional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction as used in the present context are intended to encompass the multiplicity of prior-art methods for introducing heterologous nucleic acids (for example DNA) into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed: Gartland and Davey, Humana Press, Totowa, New Jersey.

The term "nucleic acid (molecule)" as used herein comprises in an advantageous embodiment additionally the untranslated sequence located at the 3' end and at the 5' end of the coding gene region: at least 500, preferably 200, particularly preferably 100 nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, particularly preferably 20 nucleotides of the sequence downstream of the 3' end of the coding gene region. An "isolated" nucleic acid molecule is separated from other nucleic acid

molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably has no sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid is derived (e.g. sequences located at the 5' and 3' ends of the nucleic acid). In various embodiments, the isolated Δ6-desaturase, Δ6-elongase or Δ5-desaturase and, if appropriate, the ω3-desaturase or Δ4-desaturase molecule used in the process may for example comprise less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived.

The nucleic acid molecules used in the process can be isolated by using standard techniques of molecular biology and the sequence information provided herein. It is also possible for example with the aid of comparative algorithms to identify a homologous sequence or homologous, conserved sequence regions at the DNA or amino acid level. These can be used as hybridization probe in standard hybridization techniques (as described for example in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for isolating further nucleic acid sequences useful in the process. The nucleic acid molecule used in the process, or parts thereof, can moreover be isolated by polymerase chain reaction, in which case oligonucleotide primers based on this sequence or on parts thereof are used (e.g. a nucleic acid molecule comprising the complete sequence or a part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been constructed on the basis of this identical sequence). For example, mRNA can be isolated from cells (e.g. by the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA can be prepared with the aid of reverse transcriptase (e.g. Moloney MLV reverse transcriptase obtainable from Gibco/BRL, Bethesda, MD or AMV reverse transcriptase, obtainable from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligo-nucleotide primers for amplification by means of polymerase chain reaction can be constructed on the basis of one of the sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 or with the aid of the amino acid sequences depicted in SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78. A nucleic acid of the invention can be amplified by standard PCR amplification techniques using cDNA or alternatively genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified in this way can be cloned into a suitable vector and characterized by DNA sequence analysis.

Oligonucleotides can be prepared by standard synthetic methods, for example using an automatic DNA synthesizer.

Homologs of the Δ5-elongase, ω3-desaturase, Δ6-desaturase, Δ6-elongase, Δ4-desaturase or Δ5-desaturase nucleic acid sequences used, having the sequence SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, mean for example allelic variants having at least about 40, 50 or 60%, preferably at least about 60 or 70%, more preferably at least about 70 or 80%, 90% or 95% and even more preferably at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity or homology to one of the nucleotide sequences shown in SEQ ID NO: 64, 66, 68 or 70, to one of the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41, to one of the nucleotide sequences shown in SEQ ID NO: 171, 173, 175, 177, 179, 181 or 183, to one of the nucleotide sequences shown in SEQ ID NO: 51, 53 or 55, to one of the nucleotide sequences shown in SEQ ID NO: 193 or 195 or to one of the nucleotide sequences shown in or SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, especially the nucleotide sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, or their homologs, derivatives or analogs or parts thereof. Also included are isolated nucleic acid molecules of a nucleotide sequence which hybridize for example under stringent conditions to one of the nucleotide sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 or a part thereof. A part means in this connection according to the invention that at least 25 base pairs (= bp), 50 bp, 75 bp, 100 bp, 125 bp or 150 bp, preferably at least 175 bp, 200 bp, 225 bp, 250 bp, 275 bp or 300 bp, particularly preferably 350 bp, 400 bp, 450 bp, 500 bp or more base pairs are used for the hybridization. It is also possible advantageously to use the complete sequence. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from the sequence depicted in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, but where the enzyme activity of the proteins encoded thereby is substantially retained for the insertion.

Nucleic acid molecules advantageous for the process of the invention can be isolated on the basis of their homology to the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase and/or Δ6-elongase nucleic acid sequences disclosed herein by using the

sequences or a part thereof as hybridization probe in standard hybridization techniques under stringent hybridization conditions. It is possible in this connection for example to use isolated nucleic acid molecules which are at least 15 nucleotides long and hybridize under stringent conditions with the nucleic acid molecules which comprise a nucleotide sequence of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77. It is also possible to use nucleic acid molecules having at least 25, 50, 100, 250 or more nucleotides.

The term "hybridizes under stringent conditions" as used herein is intended to describe hybridization and washing conditions under which nucleic acid sequences which are at least 60% mutually homologous normally remain hybridized together. The conditions are preferably such that sequences which are at least about 65%, preferably at least about 70% and particularly preferably at least about 75% or more mutually homologous normally remain hybridized together. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-restrictive example of stringent hybridization conditions are hybridizations in 6 x sodium chloride/sodium citrate (= SSC) at about 45°C, followed by one or more washing steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker is aware that these hybridization conditions differ depending on the type of nucleic acid and, for example organic solvents are present, in relation to the temperature and the concentration of the buffer. The temperature for example under "standard hybridization conditions" is, depending on the type of nucleic acid, between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvent, for example 50% formamide, is present in the abovementioned buffer, the temperature under standard conditions is about 42°C. The hybridization conditions for DNA:DNA hybrids are preferably for example 0.1 x SSC and 20°C to 45°C, preferably 30°C to 45°C. The hybridization conditions for DNA:RNA hybrids are preferably for example 0.1 x SSC and 30°C to 55°C, preferably 45°C to 55°C. The aforementioned hybridization temperatures are determined for example for a nucleic acid with a length of about 100 bp (= base pairs) and a G + C content of 50% in the absence of formamide. The skilled person knows how the necessary hybridization conditions can be determined on the basis of textbooks such as the abovementioned or from the following textbooks Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (editors) 1985, "Nucleic Acids Hybridization: A Practical

Approach", IRL Press at Oxford University Press, Oxford; Brown (editor) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

In order to determine the percentage of homology (= identity) of two amino acid sequences (for example one of the sequences of SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78) or of two nucleic acids (for example SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77), the sequences are written one under the other in order to be able to compare them optimally (for example, gaps may be introduced into the sequence of a protein or of a nucleic acid in order to generate optimal alignment with the other protein or the other nucleic acid). Then, the amino acid radicals or nucleotides at the corresponding amino acid positions or nucleotide positions are compared. If a position in a sequence is occupied by the same amino acid radical or the same nucleotide as the corresponding position in another sequence, then the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity"). The percentage of homology between the two sequences is a function of the number of identical positions which the sequences share (i.e. % homology = number of identical positions/total number of positions x 100). The programs and algorithms used to determine the homology are described above.

An isolated nucleic acid molecule which codes for an ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase and/or Δ6-elongase which is used in the process and which is homologous to a protein sequence of SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78 can be generated by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, so that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations may be introduced into one of the sequences of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 by standard techniques such as site-specific mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are preferably produced at one or more of the predicted nonessential amino acid residues. In a "conservative amino acid substitution" the amino acid residue is replaced by an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in

the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid residue in an ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase or Δ6-elongase is thus preferably replaced by another amino acid residue from the same side-chain family. An alternative possibility in another embodiment is to introduce the mutations randomly over the whole or a part of the ω3-desaturase-, Δ6-desaturase-, Δ5-desaturase-, Δ5-elongase-, Δ4-desaturase- or Δ6-elongase-encoding sequence, e.g. by saturation mutagenesis, and the resulting mutants can be screened for the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase or Δ6-elongase activity described herein in order to identify mutants which have retained the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase,Δ4-desaturase or Δ6-elongase activity. The encoded protein can be recombinantly expressed after the mutagenesis, and the activity of the protein can be determined for example by using the assays described herein.

The invention is illustrated in greater detail by the examples which follow, which are not to be construed as limiting. The content of all of the references, patent applications, patents and published patent applications cited in the present patent application is herewith incorporated by reference.

The following table shows the sequence identifiers as used in the priority application of February 21, 2006, with the German application number 102006008030.0, and the corresponding sequence identifiers in this subsequent application. The nucleic acid sequence identified by SEQ ID NO: 1 of the priority application corresponds for example to the nucleic acid sequence identified by SEQ ID NO: 64 of the subsequent application.

Table of concordance of sequence identifiers of the priority application and the sequence identifiers in the subsequent application:

SEQ ID NO: Priority application German application number 102006008030.0	**SEQ ID NO: this subsequent application**	**Organism**

1	64	*Ostreococcus tauri*
2	65	*Ostreococcus tauri*
3	1	*Phytium irregulare*
4	2	*Phytium irregulare*
5	171	*Traustochytrium sp.*
6	172	*Traustochytrium* sp.
7	51	*Thraustochytrium* ssp.
8	52	*Thraustochytrium* ssp.
9	193	*Phytophthora infestans*
10	194	*Phytophthora infestans*
11	77	*Traustochytrium* sp.
12	78	*Traustochytrium* sp.
13	109	*Ostreococcus tauri*
n.a.	110	*Ostreococcus tauri*
14	122	*Ostreococcus tauri*
n.a.	123	*Ostreococcus tauri*
15	143	*Ostreococcus tauri*
16	144	*Ostreococcus tauri*
17	161	*Cauliflower mosaic virus*
18	162	*Cauliflower mosaic virus*
19	163	*Thalassiosira pseudonana*
20	164	*Thalassiosira pseudonana*

Examples

Example 1: General cloning methods

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of *Escherichia coli* cells, bacterial cultures and the sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

Example 2: Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced with an ABI laser fluorescence DNA sequencer by the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74: 5463-5467). Fragments resulting from a polymerase chain reaction were sequenced and verified to avoid polymerase errors in constructs to be expressed.

Example 3: Cloning of genes from *Ostreococcus tauri*

It was possible by searching for conserved regions in an *Ostreococcus tauri* sequence database (genomic sequences) in each case a sequence coding for a protein having Δ5-elongase activity or Δ6-elongase activity. These are the following sequences:

Gene name	SEQ ID	Amino acids
OtELO1.1, (Δ6-Elongase)	SEQ ID NO: 143	292
OtELO2.1, (Δ5-Elongase)	SEQ ID NO: 109	300

OtElo2.1 shows greatest similarity to an elongase from *Danio rerio* (GenBank AAN77156; approx. 26% identity), whereas OtElo1.1 shows greatest similarity to the elongase from *Physcomitrella* (PSE) (approx. 36% identity) (alignments were carried out with the tBLASTn algorithm (Altschul et al. (1990) J. Mol. Biol. 215: 403-410)).

The cloning of the elongases was carried out as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down and resuspended in 100 µl of double-distilled water and stored at -20°C. The corresponding genomic DNAs were amplified by the PCR method. The corresponding primer pairs were selected so that they harbored the yeast consensus sequence for high-efficiency translation (Kozak (1986) Cell 44: 283-292) beside the start codon. Amplification of the OtElo DNAs was carried out in each case with 1 µl of thawed cells, 200 µM dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 µl. The conditions for the PCR were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes.

Example 4: Optimization of elongase genes from *Ostreococcus tauri*

Elongases from the organism *Ostreococcus tauri* were isolated as described in example 3. In order to achieve an increase in the content of C22 fatty acids, the sequences SEQ ID NO: 143 (Δ6-elongase) and SEQ ID NO: 109 (coding for a protein identified by SEQ ID NO: 110) (Δ5-elongase) were adapted to the codon usage in oilseed rape, flax and soybean. For this purpose, the amino acid sequence of the Δ6-elongase and of the Δ5-elongase (SEQ ID NO: 144 for the Δ6-elongase; SEQ ID NO: 65 for the Δ5-elongase) was back-translated to obtain degenerate DNA sequences. These DNA sequences were adapted by means of the GeneOptimizer program (from Geneart, Regensburg) to the codon usage in oilseed rape, soybean and flax, taking account of the natural frequency of individual codons. The optimized sequences obtained in this way, which are indicated in SEQ ID NO: 64 (Δ5-elongase) and SEQ ID NO: 122 (coding for a protein identified by SEQ ID NO: 123) (Δ6-elongase) were synthesized in vitro.

Example 5: Cloning of expression plasmids for heterologous expression in yeasts

To characterize the function of the optimized nucleic acid sequences, the open reading frames of the respective DNAs were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), resulting in the plasmids pOTE1.2 (comprising the Δ6-elongase sequence) and pOTE2.2 (comprising the Δ5-elongase sequence).

Overview of the elongase sequences cloned into the yeast vector pYES2.1/V5-His-TOPO:

Gene name	SEQ ID	Amino acids
pOTE1.1, (Δ6-elongase)	SEQ ID NO: 143	292
pOTE1.2, (Δ6-elongase)	SEQ ID NO: 122	292, codon-optimized
pOTE2.1, (Δ5-elongase)	SEQ ID NO: 109	300
pOTE2.2, (Δ5-elongase)	SEQ ID NO: 64	300, codon-optimized

The Saccharomyces cerevisiae strain 334 was transformed by electroporation (1500 V) with the vectors pOTE1.2 and pOTE2.2 and with the comparative constructs pOTE1.1 and pOTE2.1 which comprise the natural nucleic acid sequence coding for the Δ6-elongase and Δ5-elongase, respectively. A yeast transformed with the empty vector pYES2 was used as control. The transformed yeasts were selected on complete minimal medium (CMdum) agar plates with 2% glucose but without uracil. After the selection, three transformants in each case were selected for further functional expression.

To express the Ot elongases, initially precultures composed of in each case 5 ml of CMdum liquid medium with 2% (w/v) raffinose but without uracil were inoculated with the selected transformants and incubated at 30°C, 200 rpm for 2 days. 5 ml of CMdum liquid medium (without uracil) with 2% raffinose were then inoculated with the precultures to an OD_{600} of 0.05. Moreover, 0.2 mM γ-linolenic acid (GLA) was added in each case to the yeast culture transformed with pOTE1.1 and pOTE1.2. On the basis of the activity of OtELO1.1, an elongation of the γ-linolenic acid to the 20:3 fatty acid is to be expected. Respectively 0.2 mM arachidonic acid and eicosapentaenoic acid were added in each case to the yeast culture transformed with pOTE2.1 and pOTE2.2. Corresponding to the activity of OtELO2.1, it is to be expected that the fatty acids ARA and EPA will be elongated respectively to the 22:4 and 22:5 fatty acids. Expression was induced by adding 2% (w/v) galactose. The cultures were incubated at 20°C for a further 96 h.

Example 6: Expression of OtELO2.2 (as depicted in SEQ ID NO: 64) and OtELO1.2 (as in SEQ ID NO: 122) in yeasts

Yeasts transformed as in example 5 with the plasmids pYES2, pOTE1.2 and pOTE2.1 were analyzed in the following way:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0, in order to remove remaining medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acidic methanolysis. For this purpose, the cell sediments were incubated with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) dimethoxypropane at 80°C for 1 h. The FAMES were extracted by extraction twice with petroleum ether (PE). To remove underivatized fatty acids, the organic phases were washed once each with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and with 2 ml of distilled water. The PE phases were then dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C at a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with appropriate fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson (2001) Lipids 36(8): 761-766; Sayanova et al. (2001) Journal of Experimental Botany

52(360): 1581-1585, Sperling et al. (2001) Arch. Biochem. Biophys. 388(2): 293-298 and Michaelson et al. (1998) FEBS Letters 439(3): 215-218. The results of the analyses are depicted in table 1.

It was possible to confirm the appropriate activities both for pOTE1.1/pOTE1.2 and for pOTE2.1/2.2. The optimized sequence (respectively pOTE1.2 and pOTE2.2) showed activity in both cases. Synthesis of γ-linolenic acid could be increased only slightly by pOTE1.2 compared with the wild-type sequence. By contrast, it was possible to observe for pOTE2.2 surprisingly both an increase in the activity and an alteration in the specificity (table 1). In this connection, the activity for elongation of EPA had virtually doubled, while the elongation of ARA had more than quadrupled. It was thus possible with the optimization of the sequence of the Δ5-elongase from *Ostreococcus tauri* to increase the yield of the precursors of DHA 6-fold in yeast with the same amount of substrate.

Example 7: Cloning expression plasmids for the seed-specific expression in plants

The following general conditins described apply to all subsequent experiments unless described otherwise.

pBin19, pBI101, pBinAR, pGPTV, pCAMBIA or pSUN are preferably used for the following examples in accordance with the invention. An overview of the binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5: 446-451. A pGPTV derivative as described in DE10205607 was used. This vector differs from pGPTV by an additionally inserted AscI restriction cleavage site.

Starting point of the cloning procedure was the cloning vector pUC19 (Maniatis et al.). In the first step, the conlinin promoter fragment was amplified using the following primers:

Cnl1 C 5': gaattcggcgcgccgagctcctcgagcaacggttccggcggtatagagttgggtaattcga (SEQ ID NO: 200)

Cnl1 C 3': cccgggatcgatgccggcagatctccaccatttttttggtggtgat (SEQ ID NO: 201)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme EcoRI and then for 12 hours at 25ºC with the restriction enzyme SmaI. The cloning vector pUC19 was incubated in the same manner. Thereafter, the PCR product and the 2668 bp cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C was verified by sequencing.

In the next step, the OCS terminator (Genbank Accession V00088; De Greve, H., et al. (1982) J. Mol. Appl. Genet. 1 (6): 499-511) was amplified from the vector pGPVT-USP/OCS (DE 102 05 607) using the following primers:

OCS_C 5': aggcctccatggcctgctttaatgagatatgcgagacgcc (SEQ ID NO: 202)

OCS_C 3': cccgggccggacaatcagtaaattgaacggag (SEQ ID NO: 203)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25ºC with the restriction enzyme SmaI. The vector pUC19-Cn11-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_OCS was verified by sequencing.

In the next step, the Cnl1-B promoter was amplified by PCR by means of the following primers:

Cnl1-B 5': aggcctcaacggttccggcggtatag (SEQ ID NO: 204)

Cnl1-B 3': cccggggttaacgctagcgggcccgatatcggatcccatttttggtggtgattggttct (SEQ ID NO: 205)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25ºC with the restriction enzyme SmaI. The vector pUC19-Cn11-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cn11B_OCS was verified by sequencing.

In a further step, the OCS terminator for Cnl1B was inserted. To this end, the PCR was carried out using the following primers:

OCS2 5': aggcctcctgctttaatgagatatgcgagac (SEQ ID NO: 206)

OCS2 3': cccgggcggacaatcagtaaattgaacggag (SEQ ID NO: 207)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25ºC with the restriction enzyme SmaI. The vector pUC19-Cnl1C_Cnl1B_OCS was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cnl1B_OCS2 was verified by sequencing.

In the next step, the Cnl1-A promoter is amplified by PCR using the following primers:

Cnl1-B 5': aggcctcaacggttccggcggtatagag (SEQ ID NO: 208)

Cnl1-B 3': aggccttctagactgcaggcggccgcccgcatttttggtggtgattggt (SEQ ID NO: 209)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme StuI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was verified by sequencing.

In a further step, the OCS terminator for Cnl1A was inserted. To this end, the PCR was carried out with the following primers:

OCS2 5': ggcctcctgctttaatgagatatgcga (SEQ ID NO: 210)

OCS2 3': aagcttggcgcgccgagctcgtcgacggacaatcagtaaattgaacggaga (SEQ ID NO: 211)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 2 hours at 37°C with the restriction enzyme HindIII. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was incubated for 2 hours at 37°C with the restriction enzyme StuI and for 2 hours at 37°C with the restriction enzyme HindIII. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cnl1B_Cnl1A_OCS3 was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the Δ6-, Δ5-desaturase and Δ6-elongase. To this end, the Phytium irregulare Δ6-desaturase (WO02/26946) was amplified using the following PCR primers:

D6Des(Pir) 5': agatctatggtggacctcaagcctggagtg (SEQ ID NO: 212)

D6Des(Pir) 3': ccatggcccgggttacatcgctgggaactcggtgat (SEQ ID NO: 213)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and then for 2 hours at 37°C with the restriction enzyme NcoI. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and for 2 hours at 37°C with the restriction enzyme NcoI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir) was used for cloning the *Thraustochytrium* ssp. Δ5-desaturase (WO02/26946). To this end, the *Thraustochytrium* ssp. Δ5-desaturase was amplified using the following PCR primers:

D5Des(Tc) 5': gggatccatgggcaagggcagcgagggccg (SEQ ID NO: 214)

D5Des(Tc) 3': ggcgccgacaccaagaagcaggactgagatatc (SEQ ID NO: 215)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme BamHI and then for 2 hours at 37ºC with the restriction enzyme *Eco*RV. The vector pUC19-Cnl1_d6Des(Pir) was incubated for 2 hours at 37°C with the restriction enzyme BamHI and for 2 hours at 37°C with the restriction enzyme EcoRV. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was used for cloning the Physcomitrella patens Δ6-elongase (WO01/59128), for which purpose the latter was amplified using the following PCR primers:

D6Elo(Pp) 5': gcggccgcatggaggtcgtggagagattctacggtg (SEQ ID NO: 216)

D6Elo(Pp) 3': gcaaaagggagctaaaactgagtgatctaga (SEQ ID NO: 217)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme NotI and then for 2 hours at 37°C with the restriction enzyme XbaI. The vector pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was incubated for 2 hours at 37°C with the restriction enzyme NotI and for 2 hours at 37°C with the restriction enzyme XbaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was verified by sequencing.

The binary vector for the transformation of plants was prepared starting from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp). To this end, pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was incubated for 2 hours at 37°C with the restriction enzyme AscI. The vector pGPTV was treated in the same manner. Thereafter, the fragment from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) and the cleaved pGPTV vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was verified by sequencing.

A further construct, pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co), was used. To this end, the amplification was carried out with the following primers, starting from pUC19-Cnl1C_OCS:

Cnl1_OCS 5': gtcgatcaacggttccggcggtatagagttg (SEQ ID NO: 218)

Cnl1_OCS 3': gtcgatcggacaatcagtaaattgaacggaga (SEQ ID NO: 219)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was incubated for 2 hours at 37ºC with the restriction enzyme SalI. The vector pUC19 was incubated for 2 hours at 37°C with the restriction enzyme SalI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_OCS was verified by sequencing.

In a further step, the Calendula officinalis Δ12-desaturase gene (WO01/85968) was cloned into pUC19-Cnl1_OCS. To this end, d12Des(Co) was amplified with the following primers:

D12Des(Co) 5': agatctatgggtgcaggcggtcgaatgc (SEQ ID NO: 220)

D12Des(Co) 3': ccatggttaaatcttattacgatacc (SEQ ID NO: 221)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55ºC

Denaturation temperature: 1 min 94ºC

Elongation temperature: 2 min 72ºC

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme Bg/II and thereafter for 2 hours at the same temperature with NcoI. The vector pUC19-Cnl1_OCS was incubated in the same manner. Thereafter, the PCR fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_D12Des(Co) was verified by sequencing.

The plasmid pUC19-Cnl1_D12Des(Co) and the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) were incubated for 2 hours at 37°C with the restriction enzyme SalI. Thereafter, the vector fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and vector fragment were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was verified by sequencing.

The binary vector for the transformation of plants was prepared starting from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co). To this end, pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was incubated for 2 hours at 37°C with the restriction enzyme *Asc*I. The vector pGPTV was treated in the same manner. Thereafter, the fragment from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) and the cleaved pGPTV vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was verified by sequencing.

A further example of the use of seed-specific expression constructs is the Napin promoter. Preparation of these expression constructs in the vectors pGPTV or pSUN is described in Wu et al. (2005) Nat. Biotech. 23:1013-1017.

A further vector suitable for plant transformation is pSUN2. This vector was used in combination with the Gateway system (Invitrogen, Karlsruhe) in order to increase the number of expression cassettes present in the vector to more than four. For this purpose, the Gateway cassette A was inserted into the vector pSUN2 in accordance with the manufacturer's instructions, as described below:

The pSUN2 vector (1 µg) was incubated with the restriction enzyme EcoRV at 37° for 1 h. The Gateway cassette A (Invitrogen, Karlsruhe) was then ligated into the cut vector using the Rapid Ligation kit from Roche, Mannheim. The resulting plasmid was transformed into E. coli DB3.1 cells (Invitrogen). The isolated plasmid pSUN-GW was then verified by sequencing.

In the second step, the expression cassette was cut out of pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) using AscI and ligated into the likewise treated vector pSUN-GW. The plasmid obtained in this way pSUN-4G was used for further gene constructs.

For this purpose, firstly a pENTR clone was modified in accordance with the manufacturer's instructions (Invitrogen). The plasmid pENTR1A (Invitrogen) was incubated with the restriction enzyme EcoRI at 37° for 1 h and then treated with Klenow enzyme and with a 1 µM dNTP mix for 30 min, and subsequently the AscI adapter (5'-ggcgcgcc; phosphorylated

at the 5' end, double-stranded) was ligated into the pENTR1A vector. Genes were inserted as described above stepwise into the Cnl cassette in these modified and transferred via AscI into the pENTR vector, resulting in the pENTR-Cnl vector.

In a further step, the pSUN-8G construct was prepared. For this purpose, 5' and 3' primers for the genes with the SEQ ID NOs: 1, 3, 5 and 7 with the restriction cleavage sites described above and with the first and in each case last 20 nucleotides of the open reading frame were produced and amplified with the standard conditions (see above) and ligated into the pENTR-Cnl vector, which was subsequently subjected to a recombination reaction with the pSUN-4G vector in accordance with the manufacturer's instructions.

The construct pSUN-8G was prepared in this way and was transformed into *Brassica juncea* and *Brassica napus*. The seeds of the transgenic plants were analyzed by gas chromatography.

A further construct which was used for transformation of *B. juncea* and *B. napus* was the construct pSUN-9G. This construct was prepared according to Wu et al. (2005) Nat. Biotech. 23:1013-1017 with the napin promoter. In a modification of Wu et al. 2005, the coding sequence of OtELO2.2 was inserted in the described manner instead of the gene OmELO. The resulting construct pSUN-9G was then transformed into *B. juncea* and *B. napus*.

Example 8: Lipid extraction from plant material

The effect of the genetic modification in plants on the production of a desired compound (such as a fatty acid) can be determined by growing the modified plant under suitable conditions (such as those described above) and analyzing the medium and/or the cellular components for the elevated production of the desired product (i.e. of the lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various types of staining methods, enzymatic and microbiological methods and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon A. et al. (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", p. 469-714, VCH: Weinheim; Belter, P.A. et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., and Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz,

J.A., and Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. B3; Chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned methods, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940 and Browse et al. (1986) Analytic Biochemistry 152:141-145. The qualitative and quantitative analysis of lipids or fatty acids is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

In addition to measuring the end product of the fermentation, it is also possible to analyze other components of the metabolic pathways which are used for the production of the desired compound, such as intermediates and by-products, in order to determine the overall production efficiency of the compound. The analytical methods comprise measuring the amount of nutrients in the medium (for example sugars, hydrocarbons, nitrogen sources, phosphate and other ions), measuring the biomass composition and the growth, analyzing the production of conventional metabolytes of biosynthetic pathways and measuring gases which are generated during the fermentation. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, Ed., IRL Press, p. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAME, fatty acid methyl ester; GC-MS, gas liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

The unambiguous detection for the presence of fatty acid products can be obtained by analyzing recombinant organisms using analytical standard methods: GC, GC-MS or TLC, as described on several occasions by Christie and the references therein (1997, in: Advances on Lipid Methodology, Fourth Edition: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren [Gas chromatography/mass spectrometric methods], Lipide 33:343-353).

The material to be analyzed can be disrupted by sonication, grinding in a glass mill, liquid nitrogen and grinding or via other applicable methods. After disruption, the material must be centrifuged. The sediment is resuspended in distilled water, heated for 10 minutes at 100°C, cooled on ice and recentrifuged, followed by extraction for one hour at 90°C in 0.5 M sulfuric acid in methanol with 2% dimethoxypropane, which leads to hydrolyzed oil and lipid compounds, which give transmethylated lipids. These fatty acid methyl esters are extracted in petroleum ether and finally subjected to a GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) at a temperature gradient of between 170°C and 240°C for 20 minutes and 5 minutes at 240°C. The identity of the resulting fatty acid methyl esters must be defined using standards which are available from commercial sources (i.e. Sigma).

Plant material is initially homogenized mechanically by comminuting in a pestle and mortar to make it more amenable to extraction.

This is followed by heating at 100°C for 10 minutes and, after cooling on ice, by resedimentation. The cell sediment is hydrolyzed for one hour at 90°C with 1 M methanolic sulfuric acid and 2% dimethoxypropane, and the lipids are transmethylated. The resulting fatty acid methyl esters (FAMEs) are extracted in petroleum ether. The extracted FAMEs are analyzed by gas liquid chromatography using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) and a temperature gradient of from 170°C to 240°C in 20 minutes and 5 minutes at 240°C. The identity of the fatty acid methyl esters is confirmed by comparison with corresponding FAME standards (Sigma). The identity and position of the double bond can be analyzed further by suitable chemical derivatization of the FAME mixtures, for example to give 4,4-dimethoxyazolin derivatives (Christie, 1998) by means of GC-MS.

Example 9: Use of the optimized Δ5-elongase (as depicted in SEQ ID NO: 64) from *Ostreococcus tauri* for constructs for constitutive expression

Transformation vectors based on pGPTV-35S, a plasmid based on pBIN19-35S (Bevan M. (1984) Nucl. Acids Res. 18:203), were produced for the transformation of plants. For this purpose, firstly an expression cassette consisting of the promoter element CaMV35S (SEQ ID NO: 161) and the 35S terminator (SEQ ID NO: 162; Franck, A. et al. (1980) Cell 21 (1): 285-294) was assembled in a pUC vector. This entailed the promoter being inserted via the SalI/XbaI restriction cleavage sites and the terminator via the BamHI/SmaI restriction

cleavage sites. In addition, a polylinker with the XhoI cleavage site was attached to the terminator ('triple ligation'). The resulting plasmid pUC19-35S was then employed for cloning PUFA genes. In parallel, the open reading frames of the Δ6-desaturase (SEQ ID NO: 1), of the Δ5-desaturase (SEQ ID NO: 51) and Δ6-elongase (SEQ ID NO: 171) sequences were inserted via the EcoRV cleavage site into pUC19-35S vectors. The resulting plasmids pUC-D6, pUC-D5, pUC-E6(Tc) were used to construct the binary vector pGPTV-35S_D6D5E6(Tc). For this purpose, the vector pGPTV was digested with the enzyme SalI, the plasmid pUC-D6 was digested with SalI/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6 was then digested with SalI, the plasmid pUC-D5 was digested with SalI/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6-D5 was then digested once more with SalI, the plasmid pUC-E6(Tc) with SalI/XhoI, and the correct fragments were ligated. These sequential cloning steps resulted in the binary vector pGPTV-D6D5E6(Tc), which was employed for the transformation.

In a further procedure, the sequence of d6Elo(Tp) (SEQ ID NO: 163) was inserted into the vector pUC19-35S instead of the sequence d6Elo(Tc). The resulting plasmid pUC-E6(Tp) was used to prepare the binary vector pGPTV-35S_D6D5E6(Tp).

In a further procedure, the open reading frame of ω3Des (SEQ ID NO: 193) was cloned into pUC19-35S. The resulting plasmid pUC-ω3Pi was transferred via SalI/XhoI into the binary vectors pGPTV-D6D5E6(Tc) and pGPTV-D6D5E6(Tp). The resulting vectors pGPTV-D6D5E6(Tc)ω3Pi and pGPTV-D6D5E6(Tp)ω3Pi were employed for the plant transformation.

In a further procedure, the open reading frame of the optimized Δ5-elongase from *Ostreococcus tauri* (SEQ ID NO: 64) and the open reading frame of the Δ4-desaturase from Thraustochytrium sp. (SEQ ID NO: 77) was cloned into pUC19-35S. The resulting plasmids pUC-E5 and pUC-D4 were then transferred via SalI/XhoI in accordance with the above statements into the vector pGPTV-D6D5E6(Tp)ω3Pi. The resulting vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was employed for the plant transformation.

All the binary vectors were transformed into E. coli DH5α cells (Invitrogen) in accordance with the manufacturer's instructions. Positive clones were identified by PCR, and plasmid DNA was isolated (Qiagen Dneasy).

Example 10: Transformation of the constitutive binary vectors into plants

a) Generation of transgenic *Brassica napus* and *Brassica juncea* plants. The protocol for the transformation of oilseed rape plant was used (modification of Moloney et al. (1992) Plant Cell Reports 8:238-242)

The binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed in *Agrobacterium tumefaciens* C58C1:pGV2260 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Murashige and Skoog (1962) Physiol. Plant. 15: 473) supplemented with 3% sucrose (3MS medium) was used for the transformation of Orychophragmus violaceus. Petioles or hypocotyls of freshly germinated sterile plants (in each case approx. 1 cm^2) were incubated with a 1:50 agrobacterial dilution for 5-10 minutes in a Petri dish. This is followed by 3 days of coincubation in the dark at 25°C on 3MS medium supplemented with 0.8% Bacto agar. Thereafter, the cultivation was continued with 16 hours light/8 hours dark and a weekly rhythm on MS medium supplemented with 500 mg/l Claforan (cefotaxime-sodium), 50 mg/l kanamycin, 20 µM benzylaminopurine (BAP) and 1.6 g/l glucose. Growing shoots were transferred to MS medium supplemented with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan, then, after rooting, transferred into soil and, after cultivation, grown for two weeks in a controlled-environment cabinet or in the greenhouse, allowed to flower, mature seeds were harvested and analyzed for elongase expression such as Δ6-elongase activity or for Δ5- or Δ6-desaturase activity by means of lipid analyses. In this manner, lines with elevated contents of polyunsaturated C20- and C22-fatty acids were identified.

b) Generation of transgenic *Orychophragmus violaceus* plants

The protocol for the transformation of oilseed rape plants was used (modification of Moloney et al. (1992) Plant Cell Reports 8:238-242) as described under a).

To generate transgenic plants, the binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed into Agrobacterium tumefaciens C58C1:pGV2260 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed Agrobacterium colony in Murashige-Skoog medium (Murashige and Skoog (1962) Physiol. Plant, 15: 473) with 3% sucrose (3MS medium) was used to transform

Orychophragmus violaceus. Petioles or hypocotyls of freshly germinated sterile plants (each about 1 cm^2) were incubated with a 1:50 agrobacterial dilution in a Petri dish for 5-10 minutes. This is followed by coincubation on 3MS medium with 0.8% Bacto agar in the dark at 25°C for 3 days. The cultivation was then continued with 16 hours light/8 hours dark and in a weekly rhythm on MS medium with 500 mg/l Claforan (cefotaxime sodium), 15 mg/l kanamycin, 20 µM benzylaminopurine (BAP) and 1.6 g/l glucose. Growing shoots were transferred to MS medium with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan and, after rooting, transferred to soil and, after cultivation, grown for two weeks in a controlled environment cabinet or in a greenhouse, allowed to flower, and mature seeds were harvested and examined by lipid analyses for elongase expression such as Δ6-elongase activity or Δ5- or Δ6-desaturase activity. Lines with increased contents of polyunsaturated C20 and C22 fatty acids were identified in this way.

c) Transformation of *Arabidopsis thaliana* plants

The protocol of Bechthold et al. (1993) C.R. Acad. Sci. Ser. III Sci. Vie. 316: 1194-1199 was used.

To generate transgenic plants, the generated binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed into *Agrobacterium tumefaciens* C58C1:pMP90 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788) and, in accordance with the protocol of Bechthold et al. (1993), flowers of *Arabidopsis thaliana* cv. Columbia 0 were dipped in an agrobacterial solution with OD600=1.0. The procedure was repeated again two days later. Seeds from these flowers were then placed on agar plates with ½ MS, 2% sucrose and 50 mg/l kanamycin. Green seedlings were then transferred to soil.

Example 11: Analysis of plant material of transgenic *Orychophragmus* or *Arabidopsis* plants

Extraction of leaf material of transgenic *Orychophragmus violaceus* and *Arabidopsis thaliana* plants transformed with pGPTV-D6D5E6(Tp)ω3PiE5D4 and the gas chromatography analysis was carried out as described in example 8. Table 2 shows the results of the analyses. The various fatty acids are indicated in percent by weight. It was possible to show that long-chain polyunsaturated fatty acids were synthesized by both different plant species. It was

surprisingly possible with the optimized sequence of the Δ5-elongase (as depicted in SEQ ID NO: 64) from *Ostreococcus tauri* to obtain a distinctly higher yield of DHA than reported for example by Robert et al. (2005) Functional Plant Biology 32: 473-479 for *Arabidopsis thaliana* with 1.5% DHA. It was possible for the first time to achieve a synthesis of long-chain polyunsaturated fatty acids for Orychophragmus violaceus.

Example 12: Analysis of seeds of transgenic *Brassica juncea* lines

Extraction of seeds of transgenic *Brassica juncea* plants transformed with pSUN-9G, and the gas chromatography analysis was carried out as described in example 8. Table 6 shows the results of the analyses. The various fatty acids are indicated in percent area. As in Wu et al. 2005 it was possible to show the synthesis of long-chain polyunsaturated fatty acids (PUFA). Surprisingly, the use of the modified elongase sequence OtELO2.2 such as the nucleic acid sequence described by SEQ ID NO: 64 resulted in a drastic increase in the content of C22 fatty acids. In total, the seed oil contained about 8% by weight % polyunsaturated C22 fatty acids. Specifically, the content of the fatty acid docosahexaenoic acid (DHA) in the seed oil was 1.9% by weight %, representing an increase by a factor of 10 compared with Wu et al. 2005.

Example 13: Detailed analysis of the lipid classes and position analysis of leaf material from O. violaceus

About 1 g of leaf tissue was heated in 4 ml of isopropanol at 95°C for 10 minutes, homogenized by Polytron and shaken after addition of 1.5 ml of chloroform. The samples were centrifuged, the supernatant was collected, and the pellet was extracted again with isopropanol:chloroform 1:1 (v/v). The two extracts were combined, dried and dissolved in chloroform. The lipid extract was prefractionated on a silica prepsep column (Fisher Scientific, Nepean, Canada) into neutral lipids, glycolipids and phospholipids, eluting with chloroform:acetic acid 100:1 (v/v), acetone:acetic acid 100:1 (v/v) and methanol:chloroform:water 100:50:40 (v/v/v), respectively. These fractions were further fractionated on silica G-25 thin-layer chromatography plates (TLC; Macherey-Nagel, Düren, Germany). Neutral lipids were developed with hexane:diethyl ether:acetic acid (70:30:1), glycolipids with chloroform:methanol:ammonia (65:25:4 v/v/v) and phospholipids with chloroform:methanol:ammonia:water (70:30:4:1 v/v/v/v). The individual lipid classes were identified after spraying with primulin under UV light, removed by scraping off the plates

and either used for direct transmethylation or extracted by a suitable solvent for further analysis.

It was possible by the disclosed methods for the various lipid classes (neutral lipids, phospholipids and galactolipids) to be fractionated and analyzed separately. The glycolipids were additionally examined for the position of the individual fatty acids.

a) Regiospecific analysis of the triacylglycerides (TAG)

Three to five mg of the TLC-purified TAG were dried under nitrogen in a glass tube, resuspended in aqueous buffer by brief ultrasound treatment (1 M Tris pH 8; 2.2% $CaCl_2$ (w/v); 0.05% bile salts (w/v)) and incubated at 40°C for 4 minutes. After addition of 0.1 ml of a solution of pancreatic lipase (10 mg/ml in water), the samples were vigorously vortexed for 3 minutes, and the digestion was stopped by adding 1 ml of ethanol and 1.5 ml of 4 M HCl. The partly digested TAGs were extracted twice with diethyl ether, washed with water, dried and dissolved in a small volume of chloroform. Monoacylglycerols (MAG) were separated from the free fatty acids and undigested TAGs on a TLC plate as described above for neutral lipids. The point corresponding to the MAGs was analyzed by GC and represented the sn-2 position of the TAGs. The distribution of the fatty acids to the remaining sn-1 and sn-3 positions was calculated by the following formula: sn-1 + sn-3 = (TAG × 3 - MAG)/2.

This position analysis of the triacylglycerides revealed in this case that EPA and DHA are present in similar concentrations in the sn-2 and sn-1/3 positions, while ARA is to be found overall only in small amounts in the triacylglycerides, and here mainly in the sn-2 position (Tab. 3).

b) Stereospecific analysis of phospholipids

Fractionated and extracted phosphatidylglycol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were dried under N_2 and resuspended in 0.5 ml of borate buffer (0.5M, pH 7.5, containing 0.4 mM $CaCl_2$). After a brief ultrasound treatment, 5U of phospholipase A2 from the venom of Naja mossambica (Sigma P-7778) and 2 ml of diethyl ether were added and the samples were vortexed at room temperature for 2 hours. The ether phase was dried, the digestion was stopped with 0.3 ml of 1M HCl, and the reaction mixture was extracted with chloroform:methanol (2:1 v/v). The digested phospholipids were separated by TLC in chloroform:methanol:ammonia:water (70:30:4:2 v/v/v/v) and points which corresponded to the liberated free fatty acids and lysophospholipids were removed by

scraping and directly transmethylated.

Positional analysis of the phospholipids showed an accumulation of EPA and DHA in the sn-2 position of phosphatidylcholine (PC), while DHA was similarly distributed in sn-1 and sn-2 position in phosphatidylethanolamine (PE). Only traces of, or no, ARA was to be found in both phospholipids (Tab. 4). The concentrations of EPA and DHA in phosphatidylglycerol were lower than in the other investigated phospholipids, with accumulation in the sn-2 position also to be observed in this lipid class (Tab. 4, PG).

c) Stereospecific analysis of glycolipids

The galactolipids were investigated as a further polar lipid class. Galactolipids are found in the membranes of plastids and form the main components there.

TLC-purified monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were dried under nitrogen and dissolved in 0.5 ml of diethyl ether. Then 25 units of the lipase from Rhizopus arrhizus (Sigma 62305), resuspended in 2 ml of borate buffer (50 mM, pH 7.5 containing 2 mM $CaCl_2$), were added, and the samples were vortexed at room temperature for 2 hours. The ether phase was dried and the digestion was stopped by adding 0.3 ml of 1M HCl, and the lipids were extracted with 4 ml of chloroform:methanol (2:1 v/v). After drying, the digested galactolipids were in a small volume of chloroform:methanol (2:1 v/v) and developed twice on a precoated silica TLC plate, firstly with chloroform:methanol: ammonia:water (70:30:4:1 v/v/v/v) to about two thirds the height of the plate, followed by complete development in hexane:diethyl ether:acetic acid (70:30:1). The points which corresponded to the liberated free fatty acids and the lysogalactolipids were identified after spraying with primulin, scraped off and transmethylated directly for GC analysis.

It was possible to find VLCPUFA in these lipids too, with an accumulation of EPA in the sn-2 position being observed. DHA was to be found only in the digalactodiacylglycerols (DGDG) and was undetectable in the monogalactodiacylglycerols (MGDG) (Table 5). The distribution of VLCPUFA in galactolipids, a compartment in which these fatty acids were not expected, shows the dynamics of the synthesis and the later transformation. VLCPUFA in polar lipids are of particular nutritional value because they can be absorbed better in the intestines of mammals than the neutral lipids.

Table 1: Test of the optimized sequences of pOTE1.1 and pOTE2.1 in yeast. The conversion rates were determined in accordance with the substrate conversions. A distinct rise in activity was achievable with the optimized sequence in plasmid pOTE2.2.

5

Conversion rates of the *Ostreococcus tauri* elongases					
	Genes	**Substrate** **Product**	**GLA** **20:3**	**ARA** **22:4**	**EPA** **22:5**
pOTE1.1	**d6Elongase(Ot)**		**21.1**		
pOTE1.2	**d6Elongase(Ot)_opt**		**25.6**		
pOTE2.1	**d5Elongase(Ot)**			**7.3**	**35.9**
pOTE2.2	**d5Elongase(Ot)_opt**			**32.7**	**63.1**

Table 2: Gas chromatographic analysis of leaf material of *Orychophragmus violaceus* and *Arabidopsis thaliana*. The individual fatty acids are indicated in percent area.

Fatty acid composition of leaf material of *Orychophragmus violaceus*											
Fatty acids	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Control	20.9	8.5	3.3	16.0	0.0	47.4	0.0	0.0	0.0	0.0	0.0
Transgene	21.3	8.2	5.2	5.2	4.2	23.1	5.0	0.6	13.5	2.7	4.5

Fatty acid composition of leaf material of *Arabidopsis thaliana*											
Fatty acids	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Control	12.8	10.0	3.5	14.2	0.0	54.6	0.0	0.0	0.0	0.0	0.0
Transgene	19.3	8.5	5.0	4.6	6.4	31.0	4.4	0.0	6.3	1.5	6.3

Table 3: Regiospecific analysis of the triacylglycerides from leaf material from transgenic *O. violaceus* plants.

TAG	16:0	18:0	18:1n-9	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:6n-3	22:5n-3
wt	**25,12**	**3,83**	**5,06**		**18,53**		**44,72**							
sn-2	1,42	0,76	6,79		27,62		62,03							
sn-1+3	36,97	4,17	4,19		13,98		36,07							
Transgene	**22,63**	**3,12**	**3,46**	**0,77**	**2,35**	**9,51**	**6,37**	**13,83**	**0,74**	**0,83**	**3,67**	**24,96**	**2,22**	**4,15**
sn-2	1,62	0,64	8,33	1,61	5,15	16,21	10,98	19,84	0,17	1,38	1,99	24,82	3,27	3,02
sn-1+3	33,13	4,36	1,02	0,35	0,96	6,16	4,11	9,83	1,02	0,55	4,90	25,03	1,69	4,72

Table 4: Stereospecific analysis of the phospholipids from leaf material from transgenic *O. violaceus* plants.

PG	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**27,96**	**20,04**	**4,11**	**2,89**	**0,90**		**21,82**	**0,00**	**21,56**							
sn-1	17,26	0,53	2,61	3,82	1,91		39,01	0,00	34,44							
sn-2	38,66	39,56	5,62	1,96	0,00		4,62	0,00	8,69							
Transgene	**27,15**	**24,70**	**3,08**	**4,62**	**1,20**	**0,00**	**15,15**	**1,53**	**17,94**	**1,40**	**0,00**	**0,00**	**0,45**	**2,18**	**0,10**	**0,58**
sn-1	21,16	3,61	4,23	7,52	2,14		27,40	0,50	31,57	0,81			0,38	1,24	0,00	0,33
sn-2	33,15	45,79	1,94	1,71	0,27		2,90	2,57	4,30	2,00			0,51	3,13	0,27	0,83

PE	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**37,49**	**0,00**	**6,62**	**4,35**	**1,37**		**19,28**		**29,95**							
sn-1	54,22	0,00	7,74	3,39	3,42		12,64		13,71							
sn-2	20,77	0,00	5,51	5,31	0,00		25,93		46,18							
Transgene	**31,78**	**0,81**	**5,84**	**3,08**	**2,20**	**0,85**	**5,57**	**11,25**	**11,34**	**7,38**	**0,00**	**0,00**	**2,88**	**9,41**	**1,90**	**4,90**
sn-1	50,17	0,33	10,86	3,22	4,94	0,35	2,63	3,27	3,59	2,31	0,56		4,42	6,18	0,38	4,19
sn-2	13,40	1,29	0,83	2,95	0,00	1,35	8,50	19,23	19,10	12,45	0,00		1,34	12,64	3,41	5,61

PC	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
WT	**27,67**	**0,84**	**6,38**	**8,56**	**1,80**		**21,75**		**33,01**							
sn-1	48,05	0,44	8,65	5,05	3,41		14,52		18,04							
sn-2	7,28	1,24	4,11	12,06	0,18		28,97		47,98							
Transgene	**21,00**	**0,00**	**8,01**	**10,02**	**2,86**	**1,25**	**3,77**	**11,63**	**5,60**	**12,11**	**0,50**	**0,00**	**4,34**	**11,16**	**3,76**	**3,70**
sn-1	45,35	0,00	14,71	5,08	5,70	0,31	3,23	3,09	4,58	2,65	0,61	0,08	4,01	8,32	0,41	1,18
sn-2	3,36	0,00	1,30	14,96	0,02	2,20	4,31	20,18	6,62	21,56	0,38	0,00	4,66	13,99	7,12	6,22

Table 5: Stereospecific analysis of the galactolipids from leaf material from transgenic *O. violaceus* plants.

MGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**2.64**	**0.13**	**1.23**	**30.72**	**0.33**	**0.35**	**0.26**		**3.81**		**60.52**					
sn-1	0.00	0.05	0.00	7.11	0.35	0.31	0.41		4.60		87.30					
sn-2	5.34	0.21	2.55	54.34	0.31	0.39	0.12		3.01		33.74					
tr	**4.16**	**0.20**	**1.08**	**33.81**	**0.93**	**0.73**	**0.52**	**0.03**	**1.64**	**1.88**	**44.82**	**2.73**	**0.04**	**0.30**	**0.50**	**5.08**
sn-1	1.22	0.29	0.54	4.79	1.51	1.15	0.93	0.00	2.80	0.14	80.19	0.00	0.08	0.17	0.87	3.86
sn-2	7.11	0.11	1.61	62.82	0.34	0.31	0.11	0.11	0.47	3.62	9.46	5.48	0.00	0.43	0.14	6.31

DGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**17.67**	**0.19**	**0.38**	**2.15**	**1.61**	**0.51**	**0.94**		**5.55**		**70.71**					
sn-1	16.84	0.25	0.50	2.52	2.21	0.55	1.75		6.07	0.00	68.74					
sn-2	18.50	0.12	0.27	1.78	1.01	0.46	0.13		5.05		72.68					
tr	**18.50**	**0.00**	**0.00**	**2.62**	**2.84**	**1.36**	**1.39**	**0.00**	**6.28**	**3.55**	**54.66**	**0.00**	**0.00**	**0.00**	**2.18**	**5.36**
sn-1	22.74	0.17	0.23	0.48	4.55	1.71	2.32	0.24	9.22	0.23	55.06	0.27	0.00	0.00	0.36	1.23
sn-2	14.27	0.00	0.00	4.77	1.12	1.00	0.46	0.00	3.33	6.88	53.26	0.00	0.00	0.00	4.01	9.49

Table 6: Gas chromatographic determination of the fatty acids from seeds of transgenic *Brassica juncea* plants transformed with the construct pSUN-9G in percent by weight. WT describes the unmodified wild-type control.

	Lipid Profile(%)							
	16:0	18:0	18:1	18:2	γ18:3	α18:3	18:4	20:0
BJ223_PUFA184_MKP71_581A	4,4	3,0	22,5	16,9	27,0	4,9	3,2	0,6
BJ223_PUFA184_MKP71_581A	4,7	3,9	17,9	10,6	29,5	4,2	4,0	0,9
BJ223_PUFA184_MKP71_581A	4,4	3,0	18,9	13,8	30,5	4,1	3,2	0,7
BJ223_PUFA184_MKP71_581A	4,6	3,3	20,5	13,2	29,8	4,2	3,3	0,8

20:3 (8,11,14)	20:3 (11,14,17)	20:4 (ARA) (5,8,11,14)	20:4 (ETeA) (8,11,14,17)	20:5 (EPA) (5,8,11,14,17)	22:1	22:4	22:5	22:6
1,1	0,5	3,1	0,6	4,6	0,0	1,5	2,0	1,5
2,0	0,9	4,2	1,0	4,1	0,0	3,1	3,5	1,9
1,3	0,7	4,1	0,5	4,5	0,0	2,7	2,8	1,6
1,4	0,6	3,6	0,6	4,4	0,0	2,4	2,5	1,6

Electronic Acknowledgement Receipt

EFS ID:	30735300
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Hui-Ju Wu
Filer Authorized By:	
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	23-OCT-2017
Filing Date:	06-SEP-2016
Time Stamp:	18:30:55
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response_NonFinal.pdf	78880 e08bcbbe5537e3923d53d8e03c88fe049249ec20	yes	11

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Specification	2	3
	Claims	4	7
	Applicant Arguments/Remarks Made in an Amendment	8	11

Warnings:					
Information:					
2	Specification	Substitute_Specification.pdf	714422 16809a6adef0815e19d6803d27babf9641188e2b	no	86
Warnings:					
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		Total Files Size (in bytes):	793302		

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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/256,914	Filing Date 09/06/2016	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **10/23/2017**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 23	Minus	** 23	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
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The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
EVELYN NIMMONS

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	15/256,914 – Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA*	US-6,194,167-B1	2001-02-27	Browse et al.	
	AB*	US-7,211,656-B2	2007-05-01	Mukerji et al.	
	AC*	US-7,238,851-B2	2007-07-03	Kang	
	AD*	US-8,049,064-B2	2011-11-01	Cirpus et al.	
	AE*	US-8,088,974-B2	2012-01-03	Lerchl et al.	
	AF*	US-8,455,035-B2	2013-06-04	Rein et al.	
	AG*	US-8,993,841-B2	2015-03-31	Napier et al.	
	AH*	US-8,785,727-B2	2014-07-22	Bauer et al.	
	AI*	US-9,493,520-B2	2016-11-15	Bauer et al.	
	AJ*	US-2003/0163845-A1	2003-08-28	Mukerji et al.	
	AK*	US-2003/0196217-A1	2003-10-16	Mukerji et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA	WO-02/057465-A2	2002-07-25	BASF Plant Science GmbH		See US 8,088,974

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	15/256,914 – Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	"Codex Standard for Named Vegetable Oils – CX-STAN 210-1999", excerpt from Codex Alimentarius, 2001, Vol. 8, pp. 11-25.	
	CB	"Danio rerio Polyunsaturated Fatty Acid Elongase mRNA, Complete cds", Database GenBank, Accession No. AF532782, February 15, 2006.	
	CC	"Phaeodactylum tricornutum Delta 12 Fatty Acid Desaturase mRNA, Complete cds; Nuclear Gene for Microsomal Protein", Database GenBank, Accession No. AY165023, April 14, 2003.	
	CD	GIRKE, T., et al., "Identification of a Novel Δ6-Acyl-Group Desaturase by Targeted Gene Disruption in Physcomitrella patens", The Plant Journal, 1998, Vol. 15, No. 1, pp. 39-48.	
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	CM	KANG, Z. B., et al., "Adenoviral Gene Transfer of *Caenorhabditis elegans* n-3 Fatty Acid Desaturase Optimizes Fatty Acid Composition in Mammalian Cells", PNAS, 2001, Vol. 98, No. 7, pp. 4050-4054.	

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(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

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(71) Anmelder *(für alle Bestimmungsstaaten mit Ausnahme von US)*: **BASF PLANT SCIENCE GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

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[Fortsetzung auf der nächsten Seite]

(54) Title: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS, NOVEL BIOSYNTHESIS GENES AND NOVEL PLANT EXPRESSION CONSTRUCTS

(54) Bezeichnung: VERFAHREN ZUR HERSTELLUNG MEHRFACH UNGESAETTIGTER FETTSAEUREN, NEUE BIOSYNTHESEGENE SOWIE NEUE PFLANZLICHE EXPRESSIONSKONSTRUKTE

(57) Abstract: The invention relates to a method for producing unsaturated fatty acids comprising at least two double bonds, and a method for producing triglycerides having an increased content of polyunsaturated fatty acids comprising at least two double bonds. The invention also relates to the advantageous use of the nucleic acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 18 in the inventive methods, and for producing a transgenic organism, preferably a transgenic plant or a transgenic micro-organism having an increased content of fatty acids, oils or lipids containing unsaturated C_{18}, C_{20}, or C_{22} fatty acids. The invention further relates to novel desaturases comprising the sequences of SEQ ID NO. 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 11, or the homologues, derivatives or analogues thereof, and gene constructs comprising said genes or the homologues, derivatives or analogues thereof, in addition to the use of the same, either alone or combined with biosynthesis genes of polyunsaturated fatty acids, as advantageously represented in SEQ ID NO: 7 and SEQ ID NO: 9. Furthermore, the invention relates to isolated nucleic acid sequences, expression cassettes containing said nucleic acid sequences, vectors, and transgenic organisms containing at least one nucleic acid sequence or an expression cassette. The invention also relates to unsaturated fatty acids comprising at least two double bonds and triglycerides having an increased content of unsaturated fatty acids comprising at least two double bonds, and the use of the same. Finally, the invention relates to multi-expression cassettes for seed-specific expression, in addition to vectors or organisms comprising a desaturase gene, either alone or combined with other desaturases having the sequence of SEQ ID NO: 7 and/or elongase genes having the sequence of ID NO: 9 or the homologues, derivatives or analogues of the same, expressed by means of the above-mentioned expression cassettes.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung von ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen und/oder ein Verfahren zur Herstellung von Triglyceriden mit erhöhtem gehalt an mehrfach ungesättigten Fettsäuren mit mindestens höhtem Gehalt an mehrfach ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen. Die Erfindung betrifft weiterhin die vorteilhafte Verwendung der Nukleinsäuresequenzen SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 11 oder SEQ ID NO: 18 im Verfahren sowie zur Herstellung eines transgenen Organismuses bevorzugt einer transgenen Pflanze oder eines transgenen Mikroorganismus mit erhöhtem Gehalt an Fettsäuren, Ölen oder Lipiden mit ungesättigten C_{18}-, C_{20}-, oder C_{22}-Fettsäuren. Die Erfindung betrifft weiterhin neue Desaturasen mit den in den Sequenzen SEQ ID No. 1, SEQ ID NO: 3, SEQ ID NO: 5 und SEQ ID NO: 11 oder seine Homologen, Derivate oder Analoga sowie Genkonstrukte, die diese Gene oder ihre Homologen, Derivate oder Analoga umfasst sowie Ihre Verwendung allein oder in Kombination mit Biosynthesegenen polyungesätigter Fettsäuren wie vorteilhaft in SEQ ID NO: 7 und SEQ ID NO: 9 dargestellt. Ausserdem betrifft die Erfindung isolierte Nukleinsäuresequenzen; Expressionskassetten enthaltend die Nukleinesäuresequenzen. Vektoren und transgene Organismen enthaltend mindestens eine Nukleinsäuresequenz bzw. eine Expressionskassette. Ausserdem betrifft die Erfindung ungesättigte Fettsäuren mit mindestens zwei Doppelbindungen sowie Triglyceride mit einem erhöhten Gehalt an ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen und deren Verwendung.

[Fortsetzung auf der nächsten Seite]

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Die Erfindung betrifft zudem Multiexpressionskassetten zur samen-spezifischen Expression und Vektoren oder Organismen, die ein Desaturasegen allein oder in Kombination mit weiteren Desaturasen mit der Sequenz SEQ ID NO: 7 und/oder Elongasegen mit de Sequenz ID NO: 9 oder seine Homologen, Derivate oder Analoga unter Verwendung vesagter Expressionskassetten umfassen.

Verfahren zur Herstellung mehrfach ungesättigter Fettsäuren, neue Biosynthesegene sowie neue pflanzliche Expressionskonstrukte

Beschreibung

Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung von ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen und/oder ein Verfahren zur Herstellung von Triglyceriden mit erhöhtem Gehalt an mehrfach ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen. Die Erfindung betrifft weiterhin die vorteilhafte Verwendung der Nukleinsäuresequenzen SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder 11 im Verfahren sowie zur Herstellung eines transgenen Organismuses bevorzugt einer transgenen Pflanze oder eines transgenen Mikroorganismus mit erhöhtem Gehalt an Fettsäuren, Ölen oder Lipiden mit ungesättigten C_{18}-, C_{20}-, oder C_{22}-Fettsäuren.

Die Erfindung betrifft weiterhin neue Desaturasen mit den in den Sequenzen SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 und SEQ ID NO: 11 oder seine Homologen, Derivate oder Analoga sowie Genkonstrukte, die diese Gene oder ihre Homologen, Derivate oder Analoga umfasst sowie Ihre Verwendung allein oder in Kombination mit Biosynthesegenen polyungesättigter Fettsäuren wie vorteilhaft in SEQ ID NO: 7 und SEQ ID NO: 9 dargestellt.

Außerdem betrifft die Erfindung isolierte Nukleinsäuresequenzen; Expressionskassetten enthaltend die Nukleinesäuresequenzen, Vektoren und transgene Organismen enthaltend mindestens eine Nukleinsäuresequenz bzw. eine Expressionskassette. Außerdem betrifft die Erfindung ungesättigte Fettsäuren mit mindestens zwei Doppelbindungen sowie Triglyceride mit einem erhöhten Gehalt an ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen und deren Verwendung.

Die Erfindung betrifft zudem Multiexpressionskassetten zur samenspezifischen Expression und Vektoren oder Organismen, die ein Desaturasegen allein oder in Kombination mit weiteren Desaturasen mit der Sequenz SEQ ID NO:7 und/oder Elongasegenen mit der Sequenz ID NO: 9 oder seine Homologen, Derivate oder Analoga unter Verwendung besagter Expressionskassetten umfassen.

Eine Reihe von Produkten und Nebenprodukten natürlich vorkommender Stoffwechselprozesse in Mikroorganismen, tierischen und pflanzlichen Zellen sind für viele Industriezweige, einschließlich der Futtermittel-, Nahrungsmittel-, Kosmetik- und pharmazeutischen Industrie, nützlich. Zu diesen gemeinsam als

"Feinchemikalien" bezeichneten Molekülen gehören beispielsweise Lipide und Fettsäuren, unter denen eine beispielhafte Klasse die mehrfach ungesättigten Fettsäuren sind. Fettsäuren und Triglyceride haben eine Vielzahl von Anwendungen in der Lebensmittelindustrie, der Tierernährung, der Kosmetik und im Pharmabereich. Je nachdem ob es sich um freie gesättigte oder ungesättigte Fettsäuren oder um Triglyceride mit einem erhöhten Gehalt an gesättigten oder ungesättigten Fettsäuren handelt, sind sie für die unterschiedlichsten Anwendungen geeignet, so werden beispielsweise mehrfach ungesättigte Fettsäuren (polyunsaturated fatty acids, PUFAs) Babynahrung zur Erhöhung des Nährwertes zugesetzt. PUFAs haben weiterhin einen positiven Einfluss auf den Cholesterinspiegel im Blut von Menschen und eignen sich daher zum Schutz gegen Herzkrankheiten. So finden sie in verschiedenen diätischen Lebensmitteln oder Medikamenten Anwendung.

Besonders geeignete Mikroorganismen zur Herstellung von PUFAs sind Mikroorganismen wie Thraustochytrien oder Schizochytrien-Stämme, Algen wie Phaeodactylum tricornutum oder Crypthecodinium-Arten, Ciliaten, wie Stylonychia oder Colpidium, Pilze, wie Mortierella, Entomophthora oder Mucor. Durch Stammselektion ist eine Anzahl von Mutantenstämmen der entsprechenden Mikroorganismen entwickelt worden, die eine Reihe wünschenswerter Verbindungen, einschließlich PUFAs, produzieren. Die Selektion von Stämmen mit verbesserter Produktion eines bestimmten Moleküls ist jedoch ein zeitraubendes und schwieriges Verfahren.

Alternativ kann die Produktion von Feinchemikalien geeigneterweise über die Produktion von Pflanzen, die so entwickelt sind, dass sie die vorstehend genannten PUFAs herstellen, im großen Maßstab durchgeführt werden. Besonders gut für diesen Zweck geeignete Pflanzen sind Ölfruchtpflanzen, die große Mengen an Lipidverbindungen enthalten wie Raps, Canola, Lein, Soja, Sonnenblumen, Borretsch und Nachtkerze. Aber auch andere Nutzpflanzen, die Öle oder Lipide und Fettsäuren enthalten, sind gut geeignet, wie in der eingehenden Beschreibung dieser Erfindung erwähnt. Mittels herkömmlicher Züchtung ist eine Reihe von Mutantenpflanzen entwickelt worden, die ein Spektrum an wünschenswerten Lipiden und Fettsäuren, Cofaktoren und Enzymen produzieren. Die Selektion neuer Pflanzensorten mit verbesserter Produktion eines bestimmten Moleküls ist jedoch ein zeitaufwändiges und schwieriges Verfahren oder sogar unmöglich, wenn die Verbindung in der entsprechenden Pflanze nicht natürlich vorkommt, wie im Fall von mehrfach ungesättigten C_{18}-, C_{20}-Fettsäuren und C_{22}-Fettsäuren und solchen mit längeren Kohlenstoffketten.

Aufgrund der positiven Eigenschaften ungesättigter Fettsäuren hat es in der Vergangenheit nicht an Ansätzen gefehlt, Gene, die an der Synthese von Fettsäuren bzw. Triglyceriden beteiligt sind, für die Herstellung von Ölen in verschiedenen Organismen mit geändertem Gehalt an ungesättigten Fettsäuren verfügbar zu machen. So wird in WO 91/13972 und seinem US-Äquivalent eine Δ-9-Desaturase beschrieben. In WO 93/11245 wird eine Δ-15-Desaturase in WO 94/11516 wird eine Δ-12-Desaturase beansprucht. Δ-6-Desaturasen werden in WO 93/06712, US 5,614,393, WO 96/21022 und WO 99/27111 beschrieben. Weitere Desaturasen werden beispielsweise in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144-20149, Wada et al., Nature 347, 1990: 200-203 oder Huang et al., Lipids 34, 1999: 649-659 beschrieben. In WO 96/13591 wird eine Δ-6-Palmitoyl-ACP-Desaturase beschrieben und beansprucht. Die biochemische Charakterisierung der verschiedenen Desaturasen ist jedoch bisher nur unzureichend erfolgt, da die Enzyme als membrangebundene Proteine nur sehr schwer zu isolieren und charakterisieren sind (McKeon et al., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777-792).

In Hefen konnte sowohl eine Verschiebung des Fettsäurespektrums zu ungesättigten Fettsäuren hin als auch eine Steigerung der Produktivität nachgewiesen werden (siehe Huang et al., Lipids 34, 1999: 649-659, Napier et al., Biochem. J., Vol. 330, 1998: 611-614). Die Expression der verschiedenen Desaturasen in transgenen Pflanzen zeigte allerdings nicht den gewünschten Erfolg. Eine Verschiebung des Fettsäurespektrum zu ungesättigten Fettsäuren hin konnte gezeigt werden, gleichzeitig zeigte sich aber, dass die Syntheseleistung der transgenen Pflanzen stark nachließ, das heißt gegenüber den Ausgangspflanzen konnten nur geringere Mengen an Ölen isoliert werden.

Weder in Hefen noch in Pflanzen werden natürlicherweise mehrfach ungesättigte C_{20}- und/oder C_{22}-Fettsäuren mit mindestens zwei Doppelbindungen im Fettsäuremolekül wie Arachidonsäure (ARA) und/oder Eicosapentaensäure (EPA) und/oder Docosahexaensäure (DHA) hergestellt.

Nach wie vor besteht daher ein großer Bedarf an neuen Genen, die für Enzyme kodieren, die an der Biosynthese ungesättigter Fettsäuren beteiligt sind und es ermöglichen, diese in einem technischen Maßstab herzustellen. Keines der bisher bekannten biotechnologischen Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren liefert die vorgenannten Fettsäuren in wirtschaftlich nutzbaren Mengen.

Es bestand daher die Aufgabe weitere Enzyme für die Synthese mehrfach ungesättigter Fettsäuren zur Verfügung zu stellen. Und diese Enzyme gegebenenfalls mit anderen Enzymen in einem Verfahren zur Herstellung mehrfach ungesättigter Fettsäuren zu verwenden. Diese Aufgabe wurde durch das erfindungsgemäße Verfahren zur Herstellung von Fettsäureestern mit einem erhöhtem Gehalt an mehrfach ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen gelöst, dadurch gekennzeichnet, man mindestens eine Nukleinsäuresequenz ausgewählt aus der Gruppe:

a) einer Nukleinsäuresequenz mit der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die aufgrund des degenerierten genetischen Codes durch Rückübersetzung der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen erhalten werden,

c) Derivate der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Nukleinsäuresequenz, die für Polypeptide mit der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen codieren und mindestens 50 % Homologie auf Aminosäureebene aufweisen, ohne dass die enzymatische Wirkung der Polypeptide wesentlich reduziert ist,

in einen Fettsäureester produzierenden Organismus einbringt, anzieht und die dem Organismus enthaltenden Fettsäureester isoliert.

Bei den im erfindungsgemäßen Verfahren verwendeten Nukleinsäuresequenzen handelt es sich um isolierte Nukleinsäuresequenzen, die für Polypeptide mit Δ-5-, Δ-6- oder Δ-12-Desaturaseaktivität codieren.

Im erfindungsgemäßen Verfahren werden vorteilhaft Fettsäureester mit mehrfach ungestättigten C_{18}-, C_{20}- und/oder C_{22}-Fettsäuremolekülen mit mindestens zwei Doppelbindungen im Fettsäureester hergestellt. Bevorzugt enthalten diese Fettsäuremoleküle drei, vier oder fünf Doppelbindungen und führen vorteilhaft zur Synthese von Arachidonsäure (ARA), Eicosapentaensäure (EPA) oder Docosahexaensäure (DHA).

Die Fettsäureester mit mehrfach ungesättigten C_{18}-, C_{20}- und/oder C_{22}-Fettsäuremolekülen können aus den Organismen, die für die Herstellung der Fettsäureester verwendet wurden, in Form eines Öls

oder Lipids beispielsweise in Form von Verbindungen wie Sphingolipide, Phosphoglyceride, Lipide, Glycolipide, Phospholipide, Monoacylglyceride, Diacylglyceride, Triacylglyceride oder sonstige Fettsäureester, die die mehrfach ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen enthalten, isoliert werden.

Als Organismus für die Herstellung im erfindungsgemäßen Verfahren kommen prinzipell alle prokaryontischen oder eurkaryontischen Organismen wie prokaryontische oder eurkaryontische Mikroorganismen wie gram-positive oder gram-negative Bakterien, Pilze, Hefen, Algen, Ciliaten, tierische oder pflanzliche Zellen, Tiere oder Pflanzen wie Moose, zweikeimblättrige oder einkeimblättrige Pflanzen in Frage. Vorteilhaft werden Organismen im erfindungsgemäßen Verfahren verwendet, die zu den Öl-produzierenden Organismen gehören, das heißt die für die Herstellung von Ölen verwendet werden, wie Mikroorganismen wie Crypthecodinium, Thraustochytrium, Phaeodactylum und Mortierella, Entomophthora, Mucor, Crypthecodinium sowie andere Algen oder Pilze sowie Tiere oder Pflanzen, insbesondere Pflanzen bevorzugt Ölfruchtpflanzen, die große Mengen an Lipidverbindungen enthalten, wie Sojabohne, Erdnuss, Raps, Canola, Sonnenblume, Safflor, Nachtkerze, Lein, Soja, Borretsch, Bäume (Ölpalme, Kokosnuss) oder Feldfrüchte, wie Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Baumwolle, Maniok, Pfeffer, Tagetes, Solanaceen-Pflanzen, wie Kartoffel, Tabak, Aubergine und Tomate, Vicia-Arten, Erbse, Alfalfa oder Buschpflanzen (Kaffee, Kakao, Tee), Salix-Arten, Bäume (Ölplame, Kokosnuß) sowie ausdauernde Gräser und Futterfeldfrüchte. Besonders bevorzugte erfindungsgemäße Pflanzen sind Ölfruchtpflanzen, wie Soja, Erdnuß, Raps, Canola, Lein, Nachtkerze, Sonnenblume, Safflor oder Bäume (Ölpalme, Kokosnuß).

Das erfindungsgemäße Verfahren beinhaltet entweder die Züchtung eines geeigneten transgenen Organismus bzw. transgenen Mikroorganismus oder die Züchtung von transgenen Pflanzenzellen, -geweben, -organen oder ganzen Pflanzen, umfassend die erfindungsgemäßen Nukleotidsequenzen der SEQ ID NO: 1, 3, 5 oder 11 gegebenenfalls in Verbindungen mit den in SEQ ID NO: 7 und/oder SEQ ID NO: 9 dargestellten Sequenzen allein oder in Kombination mit Sequenzen von Expressionskonstrukten aus SEQ ID NO: 13-17 oder ihre Homologen, Derivate oder Analoga oder ein Genkonstrukt, das die SEQ ID NO: 1, 3, 5 oder 11 ggf. in Verbindung mit SEQ ID NO: 7 und/oder 9 oder ihre Homologen, Derivate oder Analoga umfasst, oder einen Vektor, der diese Sequenz oder das Genkonstrukt umfasst, welches die Expression erfindungsgemäßer Nukleinsäuremoleküle herbeiführt, so dass eine Feinchemikalie produziert wird. Bei einer bevorzugten Ausführungsform

umfasst das Verfahren ferner den Schritt des Gewinnens einer Zelle, die eine solche erfindungsgemäße Nukleinsäuresequenzen enthält, wobei eine Zelle mit einer Desaturasenukleinsäuresequenz, einem Genkonstrukt oder einem Vektor, welche die Expression einer erfindungsgemäßen Desaturasenukleinsäure allein oder in Kombination herbeiführen, transformiert wird. Bei einer weiteren bevorzugten Ausführungsform umfasst dieses Verfahren ferner den Schritt des Gewinnens der Feinchemikalie aus der Kultur. Bei einer besonders bevorzugten Ausführungsform gehört die Zelle zur Ordnung der Ciliaten, zu Mikroorganismen, wie Pilzen, oder zum Pflanzenreich, insbesondere zu Ölfruchtpflanzen, besonders bevorzugt sind Mikroorganismen oder Ölfruchtpflanzen beispielsweise Erdnuss, Raps, Canola, Lein, Soja, Safflower (Distel), Sonnenblumen oder Borretsch.

Unter transgen im Sinne der Erfindung ist zu verstehen, daß die erfindungsgemäßen Nukleinsäuren nicht an ihrer natürlichen Stelle im Genom eines Organismus sind, dabei können die Nukleinsäuren homolog oder heterolog exprimiert werden. Tansgen bedeutet aber auch, dass die erfindungsgemäßen Nukleinsäuren an ihrem natürlichen Platz im Genom eines Organismus sind, dass jedoch die Sequenz gegenüber der natürlichen Sequenz verändert wurde und/oder das die Regulationssequenzen, der natürlichen Sequenzen verändert wurden. Bevorzugt ist unter transgen die Expression der erfindungsgemäßen Nukleinsäuren an nicht natürlicher Stelle im Genom zu verstehen, das heißt eine homologe oder bevorzugt heterologe Expression der Nukleinsäuren liegt vor. Bevorzugte transgene Organismen sind die oben genannten transgenen Pflanzen bevorzugt Ölfruchtpflanzen.

Aus den im erfindungsgemäßen Verfahren hergestellten Fettsäureestern lassen sich die enthaltenden mehrfach ungesättigten Fettsäuren beispielsweise über eine Alkalibehandlung wie wässrige KOH oder NaOH vorteilhaft in Gegenwart eines Alkohols wie Methanol oder Ethanol freisetzen und isolieren über beispielsweise Phasentrennung und anschließender Ansäuerung über z.B. H_2SO_4.

Ein weiterer Gegenstand der Erfindung sind Öle, Lipide und/oder Fettsäuren, die mindestens zwei Doppelbindungen in den Fettsäuremolekülen bevorzugt drei, vier, fünf oder sechs Doppelbindungen enthalten, die nach dem oben beschriebenen erfindungsgemäßen Verfahren hergestellt wurden. Auch sind Zusammensetzungen, die die genannten Öl-, Lipid- und/oder Fettsäuren enthalten, sowie die Verwendung der Öle, Lipide und/oder Fettsäuren oder der Zusammensetzungen in Futter, Nahrungsmitteln, Kosmetika oder Pharmazeutika ein weiterer Erfindungsgegenstand.

Ein weiterer Aspekt der Erfindung betrifft Verfahren zur Modulation der Produktion eines Moleküls durch einen Mikroorganismus. Diese Verfahren umfassen das Zusammenbringen der Zelle mit einer Substanz, welche die erfindungsgemäßen Desaturaseaktivität allein oder in Kombination oder die Desaturasenukleinsäureexpression moduliert, so dass eine zellassoziierte Aktivität relativ zu der gleichen Aktivität in Abwesenheit der Substanz verändert wird. Bei einer bevorzugten Ausführungsform wird/werden ein oder zwei Stoffwechselweg(e) der Zelle für Lipide und Fettsäuren, Cofaktoren und Enzyme moduliert oder der Transport von Verbindungen über diese Membranen moduliert, so dass die Ausbeute oder die Rate der Produktion einer gewünschten Feinchemikalie durch diesen Mikroorganismus verbessert ist. Die Substanz, welche die Desaturaseaktivität moduliert, kann eine Substanz sein, welche die Desaturaseaktivität oder Desaturasenukleinsäureexpression stimuliert oder die als Zwischenprodukt bei der Fettsäurebiosynthese verwendet werden kann. Beispiele für Substanzen, welche die Desaturaseaktivität oder Desaturasenukleinsäureexpression stimulieren, sind u.a. kleine Moleküle, aktive Desaturasen sowie desaturasenkodierende Nukleinsäuren, die in die Zelle eingebracht worden sind. Beispiele für Substanzen, welche die Desaturaseaktivität oder -Expression hemmen, sind u.a. kleine Moleküle und Antisense- Desaturasenukleinsäuremoleküle.

Ein weiterer Aspekt der Erfindung betrifft Verfahren zur Modulation der Ausbeuten einer gewünschten Verbindung aus einer Zelle, umfassend das Einbringen eines Wildtyp- oder Mutanten-Desaturasegens, das entweder auf einem separaten Plasmid gehalten oder in das Genom der Wirtszelle integriert wird, in eine Zelle. Bei Integration in das Genom kann die Integration zufallsgemäß sein oder durch derartige Rekombination erfolgen, dass das native Gen durch die eingebrachte Kopie ersetzt wird, wodurch die Produktion der gewünschten Verbindung durch die Zelle moduliert wird, oder durch Verwendung eines Gens in trans, so dass das Gen mit einer funktionellen Expressionseinheit, welche mindestens eine die Expression eines Gens gewährleistende Sequenz und mindestens eine die Polyadenylierung eines funktionell transkribierten Gens gewährleistende Sequenz enthält, funktionell verbunden ist.

Bei einer bevorzugten Ausführungsform sind die Ausbeuten modifiziert. Bei einer weiteren bevorzugten Ausführungsform wird die gewünschte Chemikalie vermehrt, wobei unerwünschte störende Verbindungen vermindert werden können. Bei einer besonders bevorzugten Ausführungsform ist die gewünschte Feinchemikalie ein Lipid oder eine Fettsäure, ein Cofaktor oder ein

Enzym. Bei besonders bevorzugten Ausführungsform ist diese Chemikalie eine mehrfach ungesättigte Fettsäure. Stärker bevorzugt ist sie ausgewählt aus Arachidonsäure (ARA), Eicosapentaensäure (EPA) oder Docosahexaensäure (DHA).

Die vorliegende Erfindung stellt neue Nukleinsäuremoleküle bereit, die zur Identifizierung und Isolierung von Desaturasen der PUFA-Biosynthese geeignet sind und zur Modifikation von Ölen, Fettsäuren, Lipiden, von Lipiden stammenden Verbindungen und am stärksten bevorzugt zur Herstellung mehrfach ungesättigter Fettsäuren verwendet werden können.

Ferner stellt die Erfindung Multiexpressionskassetten und Konstrukte zur multiparallelen samenspezifischen Expression von Genkombinationen in Pflanzen zur Verfügung.

Mikroorganismen, wie Crypthecodinium, Thraustochytrium, Phaeodactylum und Mortierella, Entomophthora, Mucor, Crypthecodinium sowie andere Algen und Pilze und Pflanzen, insbesondere Ölfruchtpflanzen sind bevorzugte Organismen für das erfindungsgemäße Verfahren.

In WO 98/01572, oder in Falciatore et al., 1999, Marine Biotechnology 1(3):239-251, sowie Dunahay et al., 1995, Genetic transformation of diatoms, J. Phycol. 31:10004-1012 und den Zitaten darin werden Klonierungsvektoren und Techniken zur genetischen Manipulation der obengenannten Mikroorganismen und Ciliaten, Algen oder verwandten Organismen, wie Phaeodactylum tricornutum, beschrieben. Dadurch können die oben genannten Nukleinsäuremoleküle im erfindungsgemäßen Verfahren, indem die Organismen zur gentechnologisch verändert werden, verwendet werden, so dass sie bessere oder effizientere Produzenten einer oder mehrerer Feinchemikalien werden. Diese verbesserte Produktion oder Effizienz der Produktion einer Feinchemikalie kann durch eine direkte Wirkung der Manipulation eines erfindungsgemäßen Gens oder durch eine indirekte Wirkung dieser Manipulation hervorgerufen werden. Unter Feinchemikalien sind im Sinne der Erfindung Fettsäureester, die mehrfach ungesättigte Fettsäuren mit mindestens zwei Doppelbindungen enthalten wie Sphingolipide, Phosphoglyceride, Lipide, Glycolipide, Phospholipide, Monoacylglyceride, Diacylglyceride, Triacylglyceride oder sonstige Fettsäureester, die die mehrfach ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen enthalten, zu verstehen. Weiter sind darunter zu verstehen Verbindungen wie Vitamine beispielsweise Vitamin E, Vitamin C, Vitamin B2, Vitamin B6, Pantolacton, Carotinoide wie Astaxanthin, β-Carotin, Zeaxanthin und andere.

Moose und Algen sind die einzigen bekannten Pflanzensysteme, die erhebliche Mengen an mehrfach ungesättigten Fettsäuren, wie Arachidonsäure (ARA) und/oder Eicosapentaensäure (EPA) und/oder Docosahexaensäure (DHA) herstellen. Moose enthalten PUFAs in Membranlipiden während Algen, algenverwandte Organismen und einige Pilze auch nennenswerte Mengen an PUFAs in der Triacylglycerolfraktion akkumulieren. Daher eignen sich Nukleinsäuremoleküle, die aus solchen Stämmen isoliert werden, die PUFAs auch in der Triacylglycerolfraktion akkumulieren, besonders vorteilhaft zur Modifikation des Lipid- und PUFA-Produktionssystems in einem Wirt, insbesondere in Mikroorganismen, wie den vorstehend erwähnten Mikroorganismen, und Pflanzen, wie Ölfruchtpflanzen, beispielsweise Raps, Canola, Lein, Soja, Sonnenblumen, Borretsch. Sie sind deshalb vorteilhaft im erfindungsgemäßen Verfahren verwendbar.

Weiterhin eignen sich deshalb die erfindungsgemäßen Nukleinsäuren besonders vorteilhaft zur Isolierung von Nukleinsäuren aus Triacylglycerol-akkumulierenden Mikroorganismen und zur Identifikation solcher DNA-Sequenzen und den durch sie codierten Enzyme in anderen Arten, die sich zur Modifikation der Biosynthese von Vorläufermolekülen von PUFAs in den entsprechenden Organismen eignen.

Mikroorganismen wie Crypthecodinium cohnii, Thraustochytrium und Phaeodactylum-Species sind Mikroganismen, die PUFAs wie ARA, EPA oder DHA in Triacylglycerolen akkumulieren können. Thraustochytrien sind phylogenetisch auch eng verwandt mit Schizochytrien Stämmen. Die Fähigkeit, anhand der erfindungsgemäßen Nukleinsäuren Desaturasen zu identifizieren, z.B. die Vorhersage der Substratspezifität von Enzymen, kann daher von signifikanter Bedeutung sein. Ferner können diese Nukleinsäuremoleküle als Bezugssequenzen zur Kartierung verwandter Genome oder zur Ableitung von PCR-Primern dienen.

Die erfindungsgemäßen Nukleinsäuremoleküle kodieren für Proteine, die als Desaturasen bezeichnet werden. Diese Desaturasen können beispielsweise eine Funktion ausüben, die am Stoffwechsel (z.B. an der Biosynthese oder am Abbau) von Verbindungen, die zur Lipid- oder Fettsäuresynthese notwendig sind, wie PUFAs, beteiligt sind oder am Transmembrantransport einer oder mehrerer Lipid-/Fettsäureverbindungen entweder in die oder aus der Zelle teilnehmen.

Die erfindungsgemäßen Nukleinsäuresequenzen codieren für Desaturasen, die zur Produktion langkettiger mehrfach ungesättigter Fettsäuren, vorzugsweise mit mehr als sechzehn,

achtzehn oder zwanzig Kohlenstoffatomen im Kohlenstoffgrundgerüst der Fettsäure und/oder mindestens zwei Doppelbindungen in der Kohlenstoffkette, geeignet sind, wobei eine erfindungsgemäße Nukleinsäure für ein Enzym codiert, das Doppelbindungen in die Δ-5-Position, in einem anderen Fall in die Δ-6-Position und in einem weiteren Fall in die Δ-12-Position einführen kann. Mithilfe dieser Nukleinsäuren können hohe Mengen an PUFAs in der Triacylgycerolfraktion erhalten werden. Weiterhin wurden weitere Desaturasen isoliert, die allein oder zusammen mit einer Δ-4-Desaturase für ein Verfahren zur Produktion polyungesättigter Fettsäuren genutzt werden können. Dabei ist in der Anmeldung unter dem Singular d.h. unter einem Desaturasegen oder -Protein auch der Plural d.h. die Desaturasegenen oder -Proteinen zu verstehen.

Die Herstellung einer Triensäure mit C_{18}-Kohlenstoffkette mithilfe von Desaturasen konnte bisher gezeigt werden. In diesen literaturbekannten Verfahren wurde die Herstellung von γ-Linolensäure beansprucht. Bisher konnte jedoch niemand die Herstellung sehr langkettiger mehrfach ungesättigter Fettsäuren (mit C_{20}- und längerer Kohlenstoffkette sowie von Triensäuren und höher ungesättigten Typen) allein durch modifizierte Organismen zeigen.

Zur Herstellung der erfindungsgemäßen langkettiger PUFAs müssen die mehrfach ungesättigten C_{18}-Fettsäuren zunächst durch die enzymatische Aktivität einer Elongase um mindestens zwei Kohlenstoffatome verlängert werden. Nach einer Elongationsrunde führt diese Enzymaktivität zu C_{20}-Fettsäuren, und nach zwei, drei und vier Elongationsrunden zu C_{22}-, C_{24}- oder C_{26}-Fettsäuren. Die in dieser Erfindung offenbarten Nukleinsäuresequenzen, die für verschiedene Desaturasen codieren, können im Konzert mit Elongasen zu sehr langkettigen, polyungesättigten führen. Die Aktivität der erfindungsgemäßen Desaturasen führt vorzugsweise zu C_{18}-, C_{20}- und/oder C_{22}-Fettsäuren mit mindestens zwei Doppelbindungen im Fettsäuremolekül, vorzugsweise mit drei, vier, fünf oder sechs Doppelbindungen, besonders bevorzugt zu C_{18}- und/oder C_{20}-Fettsäuren mit mindestens zwei Doppelbindungen im Fettsäuremolekül, vorzugsweise mit drei, vier oder fünf Doppelbindungen im Molekül. Die Fettsäureelongation kann durch Kombination der erfindungsgemäßen Desaturasen mit einer Elongaseaktivität erfolgen, wobei die durch die in SEQ ID NO: 9 codierte Elongase vorteilhaft verwendet werden kann. Nachdem die Verlängerung mit dem erfindungsgemäßen Enzym(en) stattgefunden hat, können weitere Desaturierungsschritte wie z.B. eine solche in Δ-5-Position erfolgen. Auch die Kombination mit anderen Elongasen wie solche, die zu einer Verlängerung von C_{18}- auf C_{20}- oder von C_{20}- auf C_{22-24} Ketten wie in WO0012720 offenbart führt, kann Verwendung finden und/oder einer

Desaturase mit Aktivität für Δ-4-Position kann vorteilhaft eingesetzt werden, um die hoch desaturierten Fettsäuren zu erhalten. Daher führen die Produkte der Desaturaseaktivitäten und der möglichen weiteren Desaturierung zu bevorzugten PUFAs mit einem höheren Desaturierungsgrad, wie Dihomo-gamma-Lonolensäure, Docosadiensäure, Arachidonsäure, ω6-Eicosatriendihomo-γ-linolensäure, Eicosapentaensäure, ω3-Eicosatriensäure, ω3-Eicosatetraensäure, Docosapentaensäure oder Docosahexaensäure. Substrate der erfindungsgemäßen Enzymaktivität sind zum Beispiel Taxolsäure; 6,9-Octadecadiensäure, Linolsäure, Pinolensäure, α-oder γ-Linolensäure oder Stearidonsäure sowie Arachidonsäure, Eicosatetraensäure, Docosapentaensäure, Eicosapentaensäure. Bevorzugte Substrate sind Linolsäure, γ-Linolensäure und/oder α-Linolensäure sowie Arachidonsäure, Eicosatetraensäure, Docosapentaensäure, Eicosapentaensäure. Besonders bevorzugt als Produkte des erfindungsgemäßen Verfahrens sind Arachidonsäure, Docosapentaensäure, Eicosapentaensäure. Die C_{18}-Fettsäuren mit mindestens zwei Doppelbindungen in der Fettsäure können durch die erfindungsgemäße enzymatische Aktivität in Form der freien Fettsäure oder in Form der Ester, wie Phospholipide, Glycolipide, Sphingolipide, Phosphoglyceride, Monoacylglyceride, Diacylglyceride oder Triacylglyceride, verlängert werden.

Für die menschliche Ernährung ist konjugierte Linolsäure "CLA" von besonderer Bedeutung. Unter CLA versteht man insbesondere Fettsäuren wie C18:2 $^{9\,cis,\ 11trans}$ oder das Isomer C18:2 $^{10trans,\ 12\,cis}$, die aufgrund menschlicher Enzymsysteme nach Aufnahme im Körper desaturiert bzw. elongiert werden können und zu gesundheitsfördernden Effekten beitragen können. Mit den erfindungsgemäßen Desaturasen (Δ-12-Desaturase) können auch solche konjugierten Fettsäuren mit wenigstens zwei Doppelbindungen im Molekül desaturiert werden und damit solche gesundheitsfördernden Fettsäuren der menschlichen Ernährung zugeführt werden. Weitere Beispiel für konjugierte Fettsäuren sind alpha-Parinarsäure, Punicasäure, Eleostearinsäure und Calendulasäure.

Unter der Verwendung von Klonierungsvektoren in Pflanzen und bei der Pflanzentransformation, wie denjenigen, die veröffentlicht sind in und dort zitiert sind: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Kapitel 6/7, S. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225)), lassen

sich die erfindungsgemäßen Nukleinsäuren zur gentechnologischen Veränderung eines breiten Spektrums an Pflanzen verwenden, so dass diese ein besserer oder effizienterer Produzent eines oder mehrerer von Lipiden hergeleiteter Produkte, wie PUFAs, wird. Diese verbesserte Produktion oder Effizienz der Produktion eines von Lipiden hergeleiteten Produktes, wie PUFAs, kann durch direkte Wirkung der Manipulation oder eine indirekte Wirkung dieser Manipulation hervorgerufen werden.

Es gibt eine Reihe von Mechanismen, durch die die Veränderung eines erfindungsgemäßen Desaturaseproteins die Ausbeute, Produktion und/oder Effizienz der Produktion einer Feinchemikalie aus einer Ölfruchtpflanze oder einem Mikroorganismus aufgrund eines veränderten Proteins direkt beeinflussen kann. Die Anzahl oder Aktivität des Desaturaseproteins oder -Gens sowie von Genkombinationen von Desaturasen und Elongasen kann erhöht sein, so dass größere Mengen dieser Verbindungen de novo hergestellt werden, weil den Organismen diese Aktivität und Fähigkeit zur Biosynthese vor dem Einbringen des entsprechenden Gens fehlte. Entsprechendes gilt für die Kombination mit weiteren Desaturasen oder Elongasen oder weiteren Enzymen aus dem Lipidstoffwechsel. Auch die Verwendung verschiedener divergenter, d.h. auf DNA-Sequenzebene unterschiedlicher Sequenzen kann dabei vorteilhaft sein bzw. die Verwendung von Promotoren zur Genexpression, die eine andere zeitliche Genexpression z.B. abhängig vom Reifegrad eines Samens oder Öl-speichernden Gewebes ermöglicht.

Durch das Einbringen eines erfindungsgemäßen Desaturasegens oder mehrerer Desaturasegene in einen Organismus allein oder in Kombination mit anderen Genen in eine Zelle kann nicht nur den Biosynthesefluss zum Endprodukt erhöhen, sondern auch die entsprechende Triacylglycerin-Zusammensetzung erhöhen oder de novo schaffen. Ebenso kann die Anzahl oder Aktivität anderer Gene, die am Import von Nährstoffen, die zur Biosynthese einer oder mehrerer Feinchemikalien (z.B. Fettsäuren, polaren und neutralen Lipiden) nötig sind, erhöht sein, so dass die Konzentration dieser Vorläufer, Cofaktoren oder Zwischenverbindungen innerhalb der Zellen oder innerhalb des Speicherkompartiments erhöht ist, wodurch die Fähigkeit der Zellen zur Produktion von PUFAs, wie im folgenden beschrieben, weiter gesteigert wird. Fettsäuren und Lipide sind selbst als Feinchemikalien wünschenswert; durch Optimierung der Aktivität oder Erhöhung der Anzahl einer oder mehrerer Desaturasen, die an der Biosynthese dieser Verbindungen beteiligt sind, oder durch Zerstören der Aktivität einer oder mehrerer Desaturasen, die am Abbau dieser Verbindungen beteiligt sind, kann es möglich sein, die Ausbeute, Produktion und/oder

Effizienz der Produktion von Fettsäure- und Lipidmolekülen aus Pflanzen oder Mikroorganismen zu steigern.

Die Mutagenese der/des erfindungsgemäßen Desaturasegene(s) kann weiterhin zu einem Desaturaseprotein mit geänderten Aktivitäten führen, welche die Produktion einer oder mehrerer gewünschter Feinchemikalien direkt oder indirekt beeinflussen. Beispielsweise kann die Anzahl oder Aktivität der/des erfindungsgemäßen Desaturasegens(e) gesteigert werden, so dass die normalen Stoffwechselabfälle oder -nebenprodukte der Zelle (deren Menge möglicherweise aufgrund der Überproduktion der gewünschten Feinchemikalie erhöht ist) effizient exportiert werden, bevor sie andere Moleküle oder Prozesse innerhalb der Zelle (welche die Lebensfähigkeit der Zelle senken würden) zerstören oder die Biosynthesewege der Feinchemikalie stören würden (wodurch die Ausbeute, Produktion oder Effizienz der Produktion der gewünschten Feinchemikalie verringert wird). Ferner können die relativ großen intrazellulären Mengen der gewünschten Feinchemikalie selbst toxisch für die Zelle sein oder Enzym-Rückkopplungsmechanismen, wie die allosterische Regulation, stören, beispielsweise könnte sie durch Steigerung der Aktivität oder Anzahl anderer stromabwärts folgender Enzyme oder Entgiftungsenzyme des PUFA-Wegs die Allokation der PUFA in die Triacylgylcerin-Fraktion steigern, man könnte die Lebensfähigkeit von Saatzellen erhöhen, was wiederum zu besserer Entwicklung von Zellen in Kultur oder zu Saaten führt, die die gewünschte Feinchemikalie produzieren. Das erfindungsgemäße Desaturasegen kann auch so manipuliert werden, dass die entsprechenden Mengen der verschiedenen Lipid- und Fettsäuremoleküle hergestellt werden. Dies kann eine einschneidende Wirkung auf die Lipidzusammensetzung der Membran der Zelle haben und erzeugt neue Öle zusätzlich zum Auftreten neusynthetisierter PUFAs. Da jeder Lipidtyp unterschiedliche physikalische Eigenschaften hat, kann eine Veränderung der Lipidzusammensetzung einer Membran die Membranfluidität erheblich verändern. Änderungen der Membranfluidität können sich auf den Transport von Molekülen über die Membran sowie auf die Unversehrtheit der Zelle auswirken, die beide eine entscheidende Wirkung auf die Produktion von Feinchemikalien besitzen. In Pflanzen können diese Änderungen überdies auch andere Merkmale, wie Toleranz gegenüber abiotischen und biotischen Stresssituationen, beeinflussen.

Biotische und abiotische Stresstoleranz ist ein allgemeines Merkmal, das man an ein breites Spektrum von Pflanzen, wie Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Sojabohne, Erdnuss, Baumwolle, Öl- und Faserlein, Raps und Canola, Lein, Maniok, Pfeffer, Sonnenblume und Tagetes, Solanaceen-Pflanzen,

wie Kartoffel, Tabak, Aubergine und Tomate, Vicia-Arten, Erbse, Alfalfa, Buschpflanzen (Kaffee, Kakao, Tee), Salix-Arten, Bäume (Ölpalme, Kokosnuss) und ausdauernde Gräser und Futterfeldfrüchte, vererben möchte. Diese Feldfrüchte sind als weitere erfindungsgemäße Ausführungsform auch bevorzugte Zielpflanzen für die Gentechnologie. Besonders bevorzugte erfindungsgemäße Pflanzen sind Ölfruchtpflanzen, wie Sojabohne, Erdnuss, Raps, Canola, Sonnenblume, Lein, Safflor, Bäume (Ölpalme, Kokosnuss) oder Feldfrüchte, wie Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Alfalfa, oder Buschpflanzen (Kaffee, Kakao, Tee).

Folglich betrifft ein Aspekt der Erfindung isolierte Nukleinsäuremoleküle (z.B. cDNAs), umfassend Nukleotidsequenzen, die eine Desaturase oder mehrere Desaturasen oder biologisch aktive Teile davon codieren, oder Nukleinsäurefragmente, die sich als Primer oder Hybridisierungssonden zum Nachweis oder zur Amplifikation desaturasekodierender Nukleinsäuren (z.B. DNA oder mRNA) eignen. Bei besonders bevorzugten Ausführungsformen umfasst das Nukleinsäuremolekül eine der in Sequenz ID NO:1 bzw 3 und 5 dargestellten Nukleotidsequenzen oder die kodierende Region oder ein Komplement einer dieser Nukleotidsequenzen. Bei anderen besonders bevorzugten Ausführungsformen umfasst das erfindungsgemäße isolierte Nukleinsäuremolekül eine Nukleotidsequenz, die an eine Nukleotidsequenz, wie in der Sequenz SEQ ID NO: 1, 3, 5 oder 11 dargestellt, oder einen Teil davon hybridisiert oder zu mindestens etwa 50 %, vorzugsweise mindestens etwa 60 %, stärker bevorzugt mindestens etwa 70 %, 80 % oder 90 % und noch stärker bevorzugt mindestens etwa 95 %, 96 %, 97 %, 98 %, 99 % oder mehr homolog dazu ist. Bei anderen bevorzugten Ausführungsformen kodiert das isolierte Nukleinsäuremolekül eine der in der Sequenz SEQ ID NO: 2, 4, 6 oder 12 dargestellten Aminosäuresequenzen. Das bevorzugte erfindungsgemäße Desaturasegen besitzt vorzugsweise auch mindestens eine der hier beschriebenen Desaturaseaktivitäten.

Bei einer weiteren Ausführungsform kodiert das isolierte Nukleinsäuremolekül ein Protein oder einen Teil davon, wobei das Protein oder der Teil davon eine Aminosäuresequenz enthält, die ausreichend homolog zu einer Aminosäuresequenz der Sequenz SEQ ID NO: 2, 4, 6 oder 12 ist, dass das Protein oder der Teil davon eine Desaturaseaktivität beibehält. Vorzugsweise behält das Protein oder der Teil davon, das/der von dem Nukleinsäuremolekül kodiert wird, die Fähigkeit, am Stoffwechsel von zum Aufbau von Zellmembranen von Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilzunehmen. Bei einer Ausführungsform ist das von dem Nukleinsäuremolekül kodierte Protein zu mindestens etwa 50 %, vorzugsweise mindestens

etwa 60 % und stärker bevorzugt mindestens etwa 70 %, 80 % oder 90 % und am stärksten bevorzugt mindestens etwa 95 %, 96 %, 97 %, 98 %, 99 % oder mehr homolog zu einer Aminosäuresequenz der Sequenz SEQ ID NO: 2, 4, 6 oder 12. Bei einer weiteren bevorzugten Ausführungsform ist das Protein ein Volllängen-Protein, das im wesentlichen in Teilen homolog zu einer gesamten Aminosäuresequenz der SEQ ID NO: 2, 4, 6 oder 12 (die von dem in SEQ ID NO: 1, 3, 5 oder 11 gezeigten offenen Leserahmen herrührt) ist.

Bei anderen Ausführungsformen umfasst die isolierte Desaturase eine Aminosäuresequenz, die zu mindestens etwa 50 % homolog zu einer der Aminosäuresequenzen der SEQ ID NO: 2, 4, 6 oder 12 ist und am Stoffwechsel von zum Aufbau von Fettsäuren in einem Mikroorganismus oder einer Pflanzenzelle notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen kann, wobei desaturierte C_{18}- oder C_{20-22}-Kohlenstoffketten mit Doppelbindungen an mindestens zwei Stellen gemeint ist.

Bei einer anderen bevorzugten Ausführungsform rührt das isolierte Nukleinsäuremolekül von Phaeodactylum tricornutum UTEX646 her und kodiert ein Protein (z.B. ein Desaturasefusionsprotein), das eine biologisch aktive Domäne enthält, die zu mindestens etwa 50 % oder mehr homolog zu einer Aminosäuresequenz der Sequenz SEQ ID NR 2, 4, 6 oder 12 ist und die Fähigkeit, am Stoffwechsel von zum Aufbau von Zellmembranen von Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilzunehmen, beibehält oder zumindest eine der Desaturierungsaktivitäten resultierend in PUFAs wie GLA, ALA, Dihomo-gamma Linolensäure, ARA, EPA oder DHA oder deren Vorläufermoleküle besitzt, und umfasst auch heterologe Nukleinsäuresequenzen, die ein heterologes Polypeptid oder regulatorische Proteine kodieren.

Alternativ kann die isolierte Desaturase eine Aminosäuresequenz umfassen, die von einer Nukleotidsequenz kodiert wird, die an eine Nukleotidsequenz der SEQ ID NO: 1, 3, 5 oder 11 hybridisiert, z.B. unter stringenten Bedingungen hybridisiert, oder zu mindestens etwa 50 %, vorzugsweise mindestens etwa 60 %, stärker bevorzugt mindestens etwa 70 %, 80 % oder 90 % und noch stärker bevorzugt mindestens etwa 95 %, 96 %, 97 %, 98 %, 99 % oder mehr homolog dazu ist. Es ist ebenfalls bevorzugt, dass die bevorzugten Desaturaseformen ebenfalls eine der hier beschriebenen Desaturaseaktivitäten besitzen.

Bei einer anderen Ausführungsform ist das isolierte Nukleinsäuremolekül mindestens 15, 25, 50, 100, 250 oder mehr Nukleotide lang und hybridisiert unter stringenten Bedingungen an ein Nuklein-

säuremolekül, das eine Nukleotidsequenz der SEQ ID NO: 1, 3, 5 oder 17 umfasst. Vorzugsweise entspricht das isolierte Nukleinsäuremolekül einem natürlich vorkommenden Nukleinsäuremolekül. Stärker bevorzugt kodiert das isolierte Nukleinsäuremolekül natürlich vorkommende Phaeodactylum-Desaturase oder einen biologisch aktiven Teil davon.

Eine weitere Ausführungsform der Erfindung sind Expressionskassetten, die die Expression der erfindungsgemäßen Nukleinsäuren mit den Sequenzen SEQ ID NO: 1, 3, 5 oder 11 in den verschiedenen Organismen wie Mikroorganismen beispielsweise Bakterien, Pilze, Hefen, Ciliaten, Algen oder tierische oder pflanzliche Zellen oder in Tieren oder Pflanzen ermöglichen.

Unter der erfindungsgemäßen Expressionskassette (= Nukleinsäurekonstrukt oder -fragment) sind die in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 genannten Sequenzen, die sich als Ergebnis des genetischen Codes und/oder deren funktionellen oder nicht funktionellen Derivate zu verstehen, die mit einem oder mehreren Regulationssignalen vorteilhafterweise zur Erhöhung der Genexpression funktionell verknüpft wurden und welche die Expression der codierenden Sequenz in der Wirtszelle steuern. Diese regulatorischen Sequenzen sollen die gezielte Expression der Gene und der Proteinexpression ermöglichen. Dies kann beispielsweise je nach Wirtsorganismus bedeuten, dass das Gen erst nach Induktion exprimiert und/oder überexprimiert wird, oder dass es sofort exprimiert und/oder überexprimiert wird. Beispielsweise handelt es sich bei diesen regulatorischen Sequenzen um Sequenzen an die Induktoren oder Repressoren binden und so die Expression der Nukleinsäure regulieren. Zusätzlich zu diesen neuen Regulationssequenzen oder anstelle dieser Sequenzen kann die natürliche Regulation dieser Sequenzen vor den eigentlichen Strukturgenen noch vorhanden sein und gegebenenfalls genetisch verändert worden sein, so dass die natürliche Regulation ausgeschaltet und die Expression der Gene erhöht wurde. Das Genkonstrukt kann aber auch einfacher aufgebaut sein, das heißt es wurden keine zusätzlichen Regulationssignale vor die Nukleinsäuresequenz oder dessen Derivate inseriert und der natürliche Promotor mit seiner Regulation wurde nicht entfernt. Stattdessen wurde die natürliche Regulationssequenz so mutiert, dass keine Regulation mehr erfolgt und/oder die Genexpression gesteigert wird. Diese veränderten Promotoren können in Form von Teilsequenzen (= Promotor mit Teilen der erfindungsgemäßen Nukleinsäuresequenzen) auch allein vor das natürliche Gen zur Steigerung der Aktivität gebracht werden. Das Genkonstrukt kann außerdem vorteilhafterweise auch eine oder mehrere sogenannte "enhancer Sequenzen" funktionell verknüpft mit dem Promotor enthalten, die eine erhöhte Expression

der Nukleinsäuresequenz ermöglichen. Auch am 3'-Ende der DNA-Sequenzen können zusätzliche vorteilhafte Sequenzen inseriert werden wie weitere regulatorische Elemente oder Terminatoren. Die Δ-5-Desaturase-/Δ-6-Desaturase und/oder Δ-12-Desaturasegene können in einer oder mehreren Kopien in der Expressionskassette (= Genkonstrukt) enthalten sein.

Die regulatorischen Sequenzen bzw. Faktoren können dabei wie oben beschrieben vorzugsweise die Genexpression der eingeführten Gene positiv beeinflussen und dadurch erhöhen. So kann eine Verstärkung der regulatorischen Elemente vorteilhafterweise auf der Transkriptionsebene erfolgen, indem starke Transkriptionssignale wie Promotoren und/oder "Enhancer" verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert wird.

Ein weiterer Aspekt der Erfindung betrifft Vektoren, z.B. rekombinante Expressionsvektoren, die mindestens ein erfindungsgemäßes Nukleinsäuremolekül enthalten, und Wirtszellen, in die diese Vektoren eingebracht worden sind, insbesondere Mikroorganismen, Pflanzenzellen, Pflanzengewebe, -organe oder ganze Pflanzen. Bei einer Ausführungsform kann eine solche Wirtszelle Feinchemikalien-Verbindungen, insbesondere PUFAs, speichern; zur Isolation der gewünschten Verbindung werden die Zellen geerntet. Die Verbindung (Öle, Lipide, Triacylglyceride, Fettsäuren) oder die Desaturase können dann aus dem Medium oder der Wirtszelle, welche bei Pflanzen Zellen sind, die Feinchemikalien enthalten oder speichern, am stärksten bevorzugt Zellen von Speichergeweben, wie Samenhüllen, Knollen, Epidermis- und Samenzellen, Endosperm oder Embyrogewebe isoliert werden.

Noch ein weiterer Aspekt der Erfindung betrifft eine genetisch veränderte transgene Pflanze, bevorzugt ein Ölfruchtpflanze, wie vorstehend erwähnt, besonders bevorzugt eine Raps- oder Leinpflanze, in die ein Desaturasegen eingebracht worden ist. Bei einer Ausführungsform ist das Genom von Raps oder Lein durch Einbringen eines erfindungsgemäßen Nukleinsäuremoleküls, das eine Wildtyp- oder mutierte Desaturasesequenz kodiert, als Transgen verändert worden. Bei einer anderen Ausführungsform ist ein endogenes Desaturasegen im Genom des Spenderorganismus Phaeodactylum Mutagenese und Detektion mittels DNA-Sequenzen funktionell zerstört worden oder mittels Antisensetechnologie reprimiert worden. Bei einer bevorzugten Ausführungsform wird Raps oder Lein auch zur Produktion einer gewünschten Verbindung, wie Lipiden und Fettsäuren, wobei PUFAs besonders bevorzugt sind, verwendet.

Bei noch einer weiteren bevorzugten Ausführungsform kann das Moos Physcomitrella patens zur Demonstration der Funktion eines Desaturasesegens unter Verwendung homologer Rekombination auf der Basis der in dieser Erfindung beschriebenen Nukleinsäuren verwendet werden.

Noch ein weiterer Aspekt der Erfindung betrifft ein isoliertes Desaturasegen oder einen Teil, z.B. einen biologisch aktiven Teil, davon. Bei einer bevorzugten Ausführungsform kann die isolierte Desaturase oder ein Teil davon am Stoffwechsel von zum Aufbau von Zellmembranen in einem Mikroorganismus oder einer Pflanzenzelle notwendigen Verbindungen oder am Transport von Molekülen über dessen/deren Membranen teilnehmen. Bei einer weiteren bevorzugten Ausführungsform ist die isolierte Desaturase oder der Teil davon ausreichend homolog zu einer Aminosäuresequenz der SEQ ID NO: 2, 4, 6 oder 12 das dieses Protein oder der Teil davon die Fähigkeit, am Stoffwechsel von zum Aufbau von Zellmembranen in Mikroorganismen oder Pflanzenzellen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilzunehmen, beibehält.

Die Erfindung stellt auch eine isolierte Präparation einer Desaturase in Form eines Rohextraktes oder als reines Protein bereit.

Das Desaturasepolypeptid oder ein biologisch aktiver Teil davon kann vorteilhaft funktionsfähig mit einem weiteren Polypeptid, das eine andere enzymatische Aktivität als die Desaturasen hat beispielsweise eine Elongase-, Acyltransferase- oder sonstige Aktivität verbunden werden, so dass ein Fusionsprotein gebildet wird. Vorteilhaft hat dieses Fusionsprotein eine Aktivität, die sich von derjenigen der Desaturase allein unterscheidet. Bei anderen bevorzugten Ausführungsform nimmt dieses Fusionsprotein am Stoffwechsel von Verbindungen, die zur Synthese von Lipiden und Fettsäuren, Cofaktoren und Enzymen in Mikroorganismen oder Pflanzen notwendig sind, oder am Transport von Molekülen über diese Membranen teil. Besonders vorteilhaft moduliert das Einbringen dieses Fusionsproteins in einer Wirtszelle die Produktion einer gewünschten Verbindung innerhalb einer und durch die Zelle. Bei einer bevorzugten Ausführungsform enthalten diese Fusionsproteine auch Δ-4-, Δ-5- oder Δ-6, Δ-8-, Δ-15, Δ-17 oder Δ-19-Desaturaseaktivitäten allein oder in Kombination. Insbesondere solche Genkombinationen sind bevorzugte Ausführungsformen, die aus SEQ ID NO: 7 oder 9 gewählt sind, bzw Teilen davon, Derivate oder ihren Homologen. Insbesondere solche Kombinationen sind bevorzugt, die die vollständige Proteinaktivität wie in SEQ ID NO: 1, 3, 5 oder 11 enthalten und in

Multiexpressionskassetten definiert durch SEQ ID NO: 13, 14, 15, 16 und 17 eingefügt zur Transformation von Pflanzen und Expression in Pflanzen geeignet sind.

Eingehende Beschreibung der Erfindung

Ein erfindungsgemäßer Gegenstand ist/sind isolierte Nukleinsäuresequenz(en), die für ein Polypeptid mit Desaturaseaktivität codiert, ausgewählt aus der Gruppe:

a) einer Nukleinsäuresequenz mit der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die aufgrund des degenerierten genetischen Codes durch Rückübersetzung der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen erhalten werden,

c) Derivate der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Nukleinsäuresequenz, die für Polypeptide mit der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen codieren und mindestens 50 % Homologie auf Aminosäureebene aufweisen, ohne daß die enzymatische Wirkung der Polypeptide wesentlich reduziert ist.

Weiterhin betrifft die Erfindung eine Aminosäuresequenz, die durch die oben genannte(n) Nukleinsäuresequenz(en) codiert werden (für die Erfindung soll der Singular den Plural und umgekehrt umfassen). Speziell betrifft die Erfindung Aminosäuresequenzen, die durch die in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellte Sequenz codiert werden.

Die vorliegende Erfindung stellt Nukleinsäuren und Proteinmoleküle mit Desaturaseaktivität bereit, die am Stoffwechsel von Lipiden und Fettsäuren, PUFA-Cofaktoren und Enzymen in dem Moos Physcomitrella patens oder am Transport lipophiler Verbindungen über Membranen beteiligt sind. Die erfindungsgemäßen Verbindungen lassen sich zur Modulation der Produktion von Feinchemikalien aus Organismen, beispielsweise Mikroorganismen, wie Ciliaten, Pilzen, Hefen, Bakterien, Algen, und/oder Pflanzen, wie Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Sojabohne, Erdnuss, Baumwolle, Linum Arten wie Öl- oder Faserlein, Brassica-Arten, wie Raps, Canola und Rübsen, Pfeffer, Sonnenblume, Borretsch, Nachtkerze und Tagetes, Solanacaen-Pflanzen, wie Kartoffel, Tabak, Aubergine und Tomate, Vicia-Arten, Erbse, Maniok, Alfalfa,

Buschpflanzen (Kaffee, Kakao, Tee), Salix-Arten, Bäume (Ölpalme, Kokosnuss) und ausdauernden Gräsern und Futterfeldfrüchten, entweder direkt (z.B. wenn die Überexpression oder Optimierung eines Fettsäurebiosynthese-Proteins einen direkten Einfluss auf die Ausbeute, Produktion und/oder Effizienz der Produktion der Fettsäure aus modifizierten Organismen hat) verwenden oder können eine indirekt Auswirkung haben, die dennoch zu einer Steigerung der Ausbeute, Produktion und/oder Effizienz der Produktion einer gewünschten Verbindung oder einer Abnahme unerwünschter Verbindungen führt (z.B. wenn die Modulation des Stoffwechsels von Lipiden und Fettsäuren, Cofaktoren und Enzymen zu Veränderungen der Ausbeute, Produktion und/oder Effizienz der Produktion oder der Zusammensetzung der gewünschten Verbindungen innerhalb der Zellen führt, was wiederum die Produktion einer oder mehrerer Feinchemikalien beeinflussen kann). Aspekte der Erfindung sind nachstehend weiter erläutert.

I. Feinchemikalien und PUFAs

Der Begriff "Feinchemikalie" ist im Fachgebiet bekannt und umfasst Moleküle, die durch einen Organismus produziert worden sind und Anwendungen in verschiedenen Industrien finden, wie, aber nicht beschränkt auf, die pharmazeutische, Landwirtschafts-, Nahrungsmittel- und Kosmetik-Industrie. Diese Verbindungen umfassen Lipide, Fettsäuren, Cofaktoren und Enzyme usw. (wie z.B. beschrieben in Kuninaka, A. (1996) Nucleotides and related compounds, S. 561-612, in Biotechnology Bd. 6, Rehm et al., Hrsgb., VCH: Weinheim und darin enthaltenen Literaturstellen), Lipide, gesättigte und ungesättigte Fettsäuren (z.B. Arachidonsäure), Vitamine und Cofaktoren (wie beschrieben in Ullmann's Encyclopedia of Industrial Chemistry, Bd. A27, Vitamins, S. 443-613 (1996) VCH: Weinheim und darin enthaltenen Literaturstellen; und Ong, A.S., Niki, E., & Packer, L. (1995) Nutrition, Lipids, Health and Disease Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia and the Society for Free Radical Research - Asien, abgehalten am 1.-3. Sept. 1994 in Penang, Malysia, AOCS Press (1995)), Enzyme und sämtliche anderen von Gutcho (1983) in Chemicals by Fermentation, Noyes Data Corporation, ISBN: 0818805086, und darin angegebenen Literaturstellen beschriebenen Chemikalien. Der Stoffwechsel und die Verwendungen bestimmter Feinchemikalien sind nachstehend weiter erläutert.

Die Kombination verschiedener Vorläufermoleküle und Biosynthese-enzyme führt zur Herstellung verschiedener Fettsäuremoleküle, was eine entscheidende Auswirkung auf die Zusammensetzung der Membran

hat. Es kann angenommen werden, dass PUFAs nicht nur einfach in Triacylglycerin, sondern auch in Membranlipide eingebaut werden.

Die Synthese von Membranen ist ein gut charakterisierter Prozess, an dem eine Anzahl von Komponenten, einschließlich Lipiden als Teil der Bilayer-Membran, beteiligt sind. Die Produktion neuer Fettsäuren, wie PUFAs, kann daher neue Eigenschaften von Membranfunktionen innerhalb einer Zelle oder eines Organismus erzeugen.

Zellmembranen dienen einer Vielzahl von Funktionen in einer Zelle. Zuerst und in erster Linie grenzt eine Membran den Inhalt einer Zelle von der Umgebung ab, wodurch sie der Zelle Integrität verleiht. Membranen können auch als Schranken gegenüber dem Einstrom gefährlicher oder unerwünschter Verbindungen und auch gegenüber dem Ausstrom gewünschter Verbindungen dienen.

Detailliertere Beschreibungen und Beteiligungen von Membranen und die beteiligten Mechanismen siehe in: Bamberg, E., et al. (1993) Charge transport of ion pumps on lipid bilayer membranes, Q. Rev. Biophys. 26:1-25; Gennis, R.B. (1989) Pores, Channels and Transporters, in: Biomembranes, Molecular Structure and Function, Springer: Heidelberg, S. 270-322; und Nikaido, H., und Saier, H. (1992) Transport proteins in bacteria: common themes in their design, *Science* 258:936-942, und den in jeder dieser Literaturstellen enthaltenen Zitaten.

Die Lipidsynthese lässt sich in zwei Abschnitte unterteilen: die Synthese von Fettsäuren und ihre Bindung an sn-Glycerin-3-Phosphat sowie die Addition oder Modifikation einer polaren Kopfgruppe. Übliche Lipide, die in Membranen verwendet werden, umfassen Phospholipide, Glycolipide, Sphingolipide und Phosphoglyceride. Die Fettsäuresynthese beginnt mit der Umwandlung von Acetyl-CoA in Malonyl-CoA durch die Acetyl-CoA-Carboxylase oder in Acetyl-ACP durch die Acetyltransacylase. Nach einer Kondensationsreaktion bilden diese beiden Produktmoleküle zusammen Acetoacetyl-ACP, das über eine Reihe von Kondensations-, Reduktions- und Dehydratisierungsreaktionen umgewandelt wird, so dass ein gesättigtes Fettsäuremolekül mit der gewünschten Kettenlänge erhalten wird. Die Produktion der ungesättigten Fettsäuren aus diesen Molekülen wird durch spezifische Desaturasen katalysiert, und zwar entweder aerob mittels molekularem Sauerstoff oder anaerob (bezüglich der Fettsäuresynthese in Mikroorganismen siehe F.C. Neidhardt et al. (1996) E. coli und Salmonella. ASM Press: Washington, D.C., S. 612-636 und darin enthaltene Literaturstellen; Lengeler et al. (Hrsgb.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, und die enthaltene Literaturstellen, sowie Magnuson, K., et al. (1993) Micro-

biological Reviews 57:522-542 und die enthaltenen Literaturstellen).

Vorläufer für die PUFA-Biosynthese sind beispielsweise Ölsäure, Linol- und Linolensäure. Diese C_{18}-Kohlenstoff-Fettsäuren müssen auf C_{20} und C_{22} verlängert werden, damit Fettsäuren vom Eicosa- und Docosa-Kettentyp erhalten werden. Mithilfe verschiedener Desaturasen, wie Enzymen, welche Δ-12-Desaturase, Δ-15-Desaturase, Δ-6-Desaturase-, Δ-5- und Δ-4-Desaturase-aktivität aufweisen, können Arachidonsäure, Eicosapentaensäure und Docosahexaensäure sowie verschiedene andere langkettige PUFAs erhalten, extrahiert und für verschiedene Zwecke bei Nahrungsmittel-, Futter-, Kosmetik- oder pharmazeutischen Anwendungen verwendet werden.

Zur Herstellung langkettiger PUFAs müssen, wie oben erwähnt, die mehrfach ungesättigten C_{18}- bzw C_{20}-Fettsäuren mehrfach desaturiert werden. Die erfindungsgemäßen Nukleinsäuresequenzen kodieren erste funktionell aktive Desaturasen aus Phyeodactylum tricornutum, einem Mikroorganismus, der PUFAs in der Triacylglycerolfraktion enthält. Mit den erfindungsgemäßen Desaturasen können Doppelbindungen in die Δ-5-, Δ-6- oder Δ-12-Position eingeführt werden. Die Aktivitäten der erfindungsgemäßen Desaturasen führt vorzugsweise zu C_{18}- + C_{20}-Fettsäuren mit mindestens zwei, drei, vier oder fünf Doppelbindungen im Fettsäuremolekül, vorzugsweise zu C_{20}-Fettsäuren mit vorteilhaft drei, vier oder fünf Doppelbindungen im Fettsäuremolekül. Die Desaturierung kann vor oder nach Elongation der entsprechenden Fettsäure erfolgen. Daher führen die Produkte der Desaturaseaktivitäten und der möglichen weiteren Desaturierung und Elongation zu bevorzugten PUFAs mit höherem Desaturierungsgrad, einschließlich einer weiteren Elongation von C_{20} zu C_{22}-Fettsäuren,zu Fettsäuren wie Linolsäure, Docosadiensäure, dihomo-γ-linolensäure, Arachidonsäure, ω6-Eicosatriendihomo-γ-linolensäure, Eicosapentaensäure, ω3-Eicosatriensäure, ω3-Eicosatetraensäure, Docosapentaensäure oder Docosahexaensäure. Substrate dieser erfindungsgemäßen Enzymaktivität sind zum Beispiel Taxolsäure, 6,9-Octadecadiensäure, Ölsäure, Linolsäure, γ-Linolensäure, Pinolensäure, α-Linolensäure, Arachidonsäure, Eicosapentaensäure oder Stearidonsäure. Bevorzugte Substrate sind Linolsäure, γ-Linolensäure und/oder α-Linolensäure, dihomo-γ-linolensäure bzw. Arachidonsäure, Eicosatetraensäure oder Eicosapentaensäure. Die C_{18}-oder C_{20}-Fettsäuren mit mindestens zwei Doppelbindungen in der Fettsäure können durch die erfindungsgemäße Enzymaktivität in Form der freien Fettsäure oder in Form der Ester, wie Phospholipide, Glykolipide, Sphingolipide, Phosphoglyceride, Monoacylglyceride, Diacylglyceride, Triacylglyceride oder sonstige Ester, verlängert werden.

Ferner müssen Fettsäuren anschließend an verschiedene Modifikationsorte transportiert und in das Triacylglycerin-Speicherlipid eingebaut werden. Ein weiterer wichtiger Schritt bei der Lipidsynthese ist der Transfer von Fettsäuren auf die polaren Kopfgruppen, beispielsweise durch Glycerin-Fettsäure-Acyltransferase (siehe Frentzen, 1998, Lipid, 100(4-5):161-166).

Veröffentlichungen über die Pflanzen-Fettsäurebiosynthese, Desaturierung, den Lipidstoffwechsel und Membrantransport von fetthaltigen Verbindungen, die Betaoxidation, Fettsäure-modifikation und Cofaktoren, Triacylglycerin-Speicherung und -Assemblierung einschließlich der Literaturstellen darin siehe in den folgenden Artikeln: Kinney, 1997, Genetic Engeneering, Hrsgb.: JK Setlow, 19:149-166; Ohlrogge und Browse, 1995, Plant Cell 7:957-970; Shanklin und Cahoon, 1998, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker, 1996, Genetic Engeneering, Hrsgb.: JK Setlow, 18:111-13; Gerhardt, 1992, Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl, 1995, Biochim. Biophys Acta 1256:181-186; Kunau et al., 1995, Prog. Lipid Res. 34:267-342; Stymne et al., 1993, in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Hrsgb.: Murata und Somerville, Rockville, American Society of Plant Physiologists, 150-158, Murphy & Ross 1998, Plant Journal. 13(1):1-16.

Vitamine, Cofaktoren und Nutraceutical wie PUFAs, umfassen eine Gruppe von Molekülen, die höhere Tiere nicht mehr synthetisieren können und somit aufnehmen müssen oder die höhere Tiere nicht mehr ausreichend selbst herstellen können und somit zusätzlich aufnehmen müssen, obwohl sie leicht von anderen Organismen, wie Bakterien, synthetisiert werden. Die Biosynthese dieser Moleküle in Organismen, die sie produzieren können, wie in Bakterien, ist im großen und ganzen charakterisiert worden (Ullmann's Encyclopedia of Industrial Chemistry, "Vitamins", Bd. A27, S. 443-613, VCH: Weinheim, 1996; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley & Sons; Ong, A.S., Niki, E., & Packer, L. (1995) "Nutrition, Lipids, Health and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia and the Society for Free Radical Research Asia, abgehalten am 1.-3. Sept. 1994 in Penang, Malaysia, AOCS Press, Champaign, IL X, 374 S).

Die oben erwähnten Moleküle sind entweder selbst biologisch aktive Moleküle oder Vorstufen biologisch aktiver Substanzen, die entweder als Elektronenüberträger oder Zwischenprodukte bei einer Vielzahl von Stoffwechselwegen dienen. Diese Verbindungen haben

neben ihrem Nährwert auch einen signifikanten industriellen Wert als Farbstoffe, Antioxidantien und Katalysatoren oder andere Verarbeitungshilfsstoffe. (Einen Überblick über Struktur, Aktivität und industrielle Anwendungen dieser Verbindungen siehe z.B. in Ullmann's Encyclopedia of Industrial Chemistry, "Vitamins", Bd. A27, S. 443-613, VCH: Weinheim, 1996). Mehrfach ungesättigte Fettsäuren haben verschiedene Funktionen und gesundheitsfördernde Wirkungen, beispielsweise bei koronarer Herzerkrankung, Entzündungsmechanismen, Kinderernährung usw. Veröffentlichungen und Literaturstellen, einschließlich darin zitierter Literaturstellen, siehe in: Simopoulos, 1999, Am. J. Clin. Nutr. 70 (3. Suppl.):560-569, Takahata et al., Biosc. Biotechnol. Biochem. 1998, 62(11):2079-2085, Willich und Winther, 1995, Deutsche Medizinische Wochenschrift 120(7):229ff.

II. Elemente und Verfahren der Erfindung

Die vorliegende Erfindung beruht unter anderem auf der Entdeckung neuer Moleküle, die hier als Desaturasenukleinsäure- und -proteinmoleküle bezeichnet werden, welche eine Wirkung auf die Produktion von Zellmembranen und Lipiden Phaeodactylum tricornutum ausüben und beispielsweise die Bewegung von Molekülen über diese Membranen beeinflussen. Bei einer Ausführungsform nehmen die Desaturasemoleküle am Stoffwechsel von zum Aufbau von Zellmembranen in Organismen, wie Mikroorganismen und Pflanzen, notwendigen Verbindungen teil oder beeinflussen indirekt den Transport von Molekülen über diese Membranen. Bei einer bevorzugten Ausführungsform hat die Aktivität der erfindungsgemäßen Desaturasemoleküle zur Regulation der Produktion von Membrankomponenten und des Membrantransports eine Auswirkung auf die Produktion der gewünschten Feinchemikalie durch diesen Organismus. Bei einer besonders bevorzugten Ausführungsform ist die Aktivität der erfindungsgemäßen Desaturasemoleküle moduliert, so dass die Ausbeute, Produktion und/oder Effizienz der Produktion der Stoffwechselwege von Mikroorganismen oder Pflanzen, welche die erfindungsgemäßen Desaturasen regulieren, moduliert sind und die Effizienz des Transport von Verbindungen durch die Membranen verändert ist, was entweder direkt oder indirekt die Ausbeute, Produktion und/oder Effizienz der Produktion einer gewünschten Feinchemikalie durch Mikroorganismen und Pflanzen moduliert.

Der Begriff "Desaturase" oder "Desaturasepolypeptid" umfasst Proteine, die an der Desaturierung von Fettsäuren teilnehmen. Beispiele für Desaturasen sind in der SEQ ID NO: 1, 3, 5, 11 oder ihren Homologen, Derivaten oder Analoga offenbart. Die Begriffe Desaturase oder Desaturasenukleinsäuresequenz(en)

umfassen Nukleinsäuresequenzen, die eine Desaturase kodieren und bei denen ein Teil eine kodierende Region und ebenfalls entsprechende 5'- und 3'-untranslatierte Sequenzbereiche sein können. Beispiele für Desaturase-Gene sind die in SEQ ID NO: 1, 3, 5 oder 11 dargestellten. Die Begriffe Produktion oder Produktivität sind im Fachgebiet bekannt und beinhalten die Konzentration des Fermentationsproduktes (zum Beispiel der gewünschten Feinchemikalie), das in einer bestimmten Zeitspanne und einem bestimmten Fermentationsvolumen gebildet wird (z.B. kg Produkt pro Stunde pro Liter). Der Begriff Effizienz der Produktion umfasst die Zeit, die zur Erzielung einer bestimmten Produktionsmenge nötig ist (z.B. wie lange die Zelle zur Aufrichtung einer bestimmten Durchsatzrate einer Feinchemikalie benötigt). Der Begriff Ausbeute oder Produkt/Kohlenstoff-Ausbeute ist im Fachgebiet bekannt und umfasst die Effizienz der Umwandlung der Kohlenstoffquelle in das Produkt (d.h. die Feinchemikalie). Dies wird gewöhnlich beispielsweise ausgedrückt als kg Produkt pro kg Kohlenstoffquelle. Durch Erhöhen der Ausbeute oder Produktion der Verbindung wird die Menge der gewonnenen Moleküle oder der geeigneten gewonnenen Moleküle dieser Verbindung in einer bestimmten Kulturmenge über einen festgelegten Zeitraum erhöht. Die Begriffe Biosynthese oder Biosyntheseweg sind im Fachgebiet bekannt und umfassen die Synthese einer Verbindung, vorzugsweise einer organischen Verbindung, durch eine Zelle aus Zwischenverbindungen, beispielsweise in einem Mehrschritt- und stark regulierten Prozess. Die Begriffe Abbau oder Abbauweg sind im Fachgebiet bekannt und umfassen die Spaltung einer Verbindung, vorzugsweise einer organischen Verbindung, durch eine Zelle in Abbauprodukte (allgemeiner gesagt, kleinere oder weniger komplexe Moleküle) beispielsweise in einem Mehrschritt- und stark regulierten Prozess. Der Begriff Stoffwechsel ist im Fachgebiet bekannt und umfasst die Gesamtheit der biochemischen Reaktionen, die in einem Organismus stattfinden. Der Stoffwechsel einer bestimmten Verbindung (z.B. der Stoffwechsel einer Fettsäure) umfasst dann die Gesamtheit der Biosynthese-, Modifikations- und Abbauwege dieser Verbindung in der Zelle, die diese Verbindung betreffen.

Bei einer anderen Ausführungsform können die erfindungsgemäßen Nukleinsäuresequenzen, die für Desaturase-Moleküle codieren, die Produktion eines gewünschten Moleküls, wie einer Feinchemikalie, in einem Mikroorganismus oder in Pflanzen modulieren. Es gibt eine Reihe von Mechanismen, durch die die Veränderung einer erfindungsgemäßen Sequenz die Ausbeute, Produktion und/oder Effizienz der Produktion einer Feinchemikalie aus einem Mikroorganismus- oder Pflanzenstamm, die dieses veränderte Protein enthalten, direkt beeinflussen kann. Die Anzahl oder Aktivität

von Desaturasen, die am Transport von Feinchemikalienmolekülen innerhalb oder aus der Zelle beteiligt sind, kann erhöht werden, so dass größere Mengen dieser Verbindungen über Membranen transportiert werden, aus denen sie leichter gewonnen und ineinander umgewandelt werden. Ferner sind Fettsäuren, Triacylglycerine und/oder Lipide selbst wünschenswerte Feinchemikalien; durch Optimierung der Aktivität oder Steigern der Anzahl einer oder mehrerer erfindungsgemäßer Desaturasen, die an der Biosynthese dieser Verbindungen beteiligt sind, oder durch Stören der Aktivität einer oder mehrerer Desaturasen, die am Abbau dieser Verbindungen beteiligt sind, kann es möglich sein, die Ausbeute, Produktion und/oder Effizienz der Produktion von Fettsäure- und Lipidmolekülen aus Organismen, wie Mikroorganismen oder Pflanzen, zu erhöhen.

Die Mutagenese des erfindungsgemäßen Nukleinsäuresequenzen kann Desaturasen mit veränderten Aktivitäten hervorbringen, welche die Produktion einer oder mehrerer gewünschter Feinchemikalien aus Mikroorganismen oder Pflanzen indirekt beeinflussen. Beispielsweise können erfindungsgemäße Desaturasen, die am Export von Abfallprodukten beteiligt sind, eine größere Anzahl oder höhere Aktivität aufweisen, so dass die normalen Stoffwechselabfälle der Zelle (deren Menge möglicherweise aufgrund der Überproduktion der gewünschten Feinchemikalie erhöht ist) effizient exportiert werden, bevor sie die Moleküle in der Zelle schädigen können (was die Lebensfähigkeit der Zelle herabsetzen würde) oder die Feinchemikalien-Biosynthesewege stören können (was die Ausbeute, Produktion oder Effizienz der Produktion einer gewünschten Feinchemikalie senken würde). Die relativ großen intrazellulären Mengen der gewünschten Feinchemikalie selbst können ferner für die Zelle toxisch sein, so dass man durch Steigern der Aktivität oder Anzahl von Transportern, die diese Verbindungen aus der Zelle exportieren können, die Lebensfähigkeit der Zelle in Kultur steigern kann, was wiederum zu einer größeren Anzahl an Zellen in der Kultur führt, welche die gewünschte Feinchemikalie produzieren. Die erfindungsgemäßen Desaturasen können auch so manipuliert werden, dass die entsprechenden Mengen unterschiedlicher Lipid- und Fettsäuremoleküle produziert werden. Dies kann eine erhebliche Auswirkung auf die Lipidzusammensetzung der Zellmembran haben. Da jeder Lipidtyp unterschiedliche physikalische Eigenschaften aufweist, kann eine Veränderung der Lipidzusammensetzung einer Membran die Membranfluidität signifikant verändern. Änderungen der Membranfluidität können den Transport von Molekülen über die Membran sowie die Integrität der Zelle beeinflussen, was jeweils eine erhebliche Auswirkung auf die Produktion von Feinchemikalien aus Mikroorganismen und Pflanzen in Fermentationskultur im großen Maßstab hat. Pflanzenmembranen

verleihen spezifische Eigenschaften, wie Toleranz gegenüber Wärme, Kälte, Salz, Trockenheit sowie Toleranz gegen Pathogene, wie Bakterien und Pilze. Daher kann die Modulation der Membrankomponenten eine grundlegende Wirkung auf die Überlebensfähigkeit der Pflanzen unter den oben genannten Stressparametern haben. Dies kann über Änderungen in Signalkaskaden oder direkt über die veränderte Membranzusammensetzung erfolgen (siehe zum Beispiel: Chapman, 1998, Trends in Plant Science, 3(11):419-426) und Signalkaskaden (siehe Wang 1999, Plant Physiology, 120:645-651) oder die Kältetoleranz, wie offenbart in WO 95/18222, beeinflussen.

Die erfindungsgemäßen isolierten Nukleinsäuresequenzen sind im Genom eines Phaeodactylum tricornutum UTEX646-Stammes enthalten, der über die Algensammlung der University of Texas, Austin verfügbar ist.

Die Nukleotidsequenz der Phaeodactylum tricornutum-cDNA und die abgeleiteten Aminosäuresequenzen der Desaturasen sind in den SEQ ID NO: 1 bis 6 sowie 11 und 12 gezeigt. Es wurden Computeranalysen durchgeführt, die diese Nukleotidsequenzen als Sequenzen klassifizieren und/oder identifizieren, die am Stoffwechsel von Zellmembrankomponenten beteiligte Proteine oder am Transport von Verbindungen über Zellmembranen beteiligte Proteine bzw. der PUFA Biosynthese codieren. EST's mit der Datenbankeingabe-NO: PT001070010R und PT001078032R durch die Erfinder stellen die erfindungsgemäßen Sequenzen in SEQ ID NO: 1 und 3 dar. Die Sequenz des Fragments aus EST PT001070010R wurde ermittelt und ist wie dargestellt in SEQ ID NO: 5. Analog ist die Sequenz des Klones PT001078032R dargestellt in SEQ ID NO: 1. Den Klonen wurden Gennamen zugewiesen. Abkürzungen bedeuten: Pp = Physcomitrella patens, Pt = Phaeodactylum tricornutum. PT001070010R aus SEQ ID NO: 5 codiert für ein neues Gen homolog zu Δ-12-Desaturase und PT001078032R codiert für eine neuartige Δ-5-Desaturase. Pt_des6 kann gemäß Beispiel 5a mittels Polymerase Kettenreaktion unter Zuhilfenahme degenerierter Oligonukleotide isoliert werden. Ein so erhaltenes Fragment kann zum Sichten einer cDNA Bank aus Phaeodactylum tricornutum isoliert werden und die codierende Region einer Phaeodactylum tricornutum Δ-6-Desaturase erhalten werden. Ein so isoliertes Gen wird in Tabelle 1 als Pt_des6 bezeichnet und ist in SEQ ID NO: 3 dargestellt. Die korrespondierenden Aminosäuresequenzen werden durch Übersetzung des genetischen Codes der Sequenz ID NO: 1, 3 und 5 erhalten und sind als SEQ ID NO: 2, 4 und 6 definiert (siehe auch Tabelle 1). Auch eine weitere Nukleinsäuresequenz, die für eine Δ-12-Desaturase codiert,

ist Tabelle 1 zu entnehmen. Sie trägt die Klon-Nummer PT001072031R.

Tabelle 1

	Genname	Klonname	Nukleinsäure SEQ ID NO:	Polypeptid SEQ ID NO:
D5 Desaturase	Pt_des5	PT001078032R	1	2
D6 Desaturase	Pt_des6	Pt_des6	3	4
D12 Desaturase	Pt_des12	PT001070010R	5	6
D6 Desaturase	Pp_des6	Pp_des6	7	8
D6 Elongase	Pp_PSE1	PP001019019F	9	10
Δ12 Desaturase	Pt des12.2	PT001072013R	11	12

Die vorliegende Erfindung betrifft auch Proteine mit einer Aminosäuresequenz, die im wesentlichen homolog zu einer Aminosäuresequenz der SEQ ID NO:2, 4, 6 oder 12 ist. Wie hier verwendet, ist ein Protein mit einer Aminosäuresequenz, die im wesentlichen homolog zu einer ausgewählten Aminosäuresequenz ist, zu mindestens etwa 50 % homolog zu der ausgewählten Aminosäuresequenz, z.B. der gesamten ausgewählten Aminosäuresequenz. Ein Protein mit einer Aminosäuresequenz, die im wesentlichen homolog zu einer ausgewählten Aminosäuresequenz ist, kann auch zu mindestens etwa 50 bis 60 %, vorzugsweise mindestens etwa 60 bis 70 % und stärker bevorzugt mindestens etwa 70 bis 80 %, 80 bis 90 % oder 90 bis 95 % und am stärksten bevorzugt mindestens etwa 96 %, 97 %, 98 %, 99 % oder mehr homolog zu einer ausgewählten Aminosäuresequenz sein.

Die erfindungsgemäße Desaturase oder der biologisch aktive Teil oder das Fragment davon kann am Stoffwechsel von Lipiden zum Aufbau von Zellmembranen oder Speicherlipiden in Mikroorganismen teilnehmen und in Kombination mit weiteren Genen, insbesondere solchen mit Elongaseaktivität zur Elongation von C_{18}-bzw C_{20-22}-PUFAs benötigten Aktivitäten beitragen, so dass C_{18}, C_{20}-, C_{22}- oder C_{24}-PUFAs sowie verwandte PUFAs erhalten werden. Dabei können erfindungsgemäße Desaturasen in Kombination mit Elongasen und anderen Desaturasen in erfindungsgemäßen Expressionskassetten kloniert werden und zur Transformation von Pflanzen mithilfe von Agrobakterium eingesetzt werden.

Verschiedene Aspekte der Erfindung sind eingehender in den folgenden Unterabschnitten beschrieben.

A. Isolierte Nukleinsäuremoleküle

Eine Ausführungsform der Erfindung sind isolierte Nukleinsäuren, die von PUFA produzierenden Mikroorganismen stammen und für Polypeptide kodieren, die C_{18}-oder C_{20-22}-Fettsäuren mit mindestens einer, zwei, drei oder vier Doppelbindungen in der Fettsäure desaturieren.

Eine weitere erfindungsgemäße Ausführungsform sind isolierte Nukleinsäuren, umfassend Nukleotidsequenzen, die für Polypeptide kodieren, die C_{18}-bzw C_{20}-Fettsäuren mit mindestens ein, zwei, drei oder vier Doppelbindungen in der Fettsäure desaturieren und sind aus der Gruppe, bestehend aus

a) einer Nukleinsäuresequenz mit der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die aufgrund des degenerierten genetischen Codes durch Rückübersetzung der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen erhalten werden,

c) Derivate der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Nukleinsäuresequenz, die für Polypeptide mit der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen codieren und mindestens 50 % Homologie auf Aminosäureebene aufweisen, ohne dass die enzymatische Wirkung der Polypeptide wesentlich reduziert ist.

Die oben genannte erfindungsgemäße Nukleinsäure stammt von Organismen, wie Ciliaten, Pilzen, Algen oder Dinoflagellaten, die PUFAs synthetisieren können, vorzugsweise von Phaeodactylum tricornutum oder nah verwandten Organismen.

Ein Aspekt der Erfindung betrifft isolierte Nukleinsäuremoleküle, die Desaturase-Polypeptide oder biologisch aktive Teile davon kodieren, sowie Nukleinsäurefragmente, die zur Verwendung als Hybridisierungssonden oder Primer zur Identifizierung oder Amplifizierung einer Desaturase-kodierenden Nukleinsäure (z.B. Desaturase-DNA) ausreichen. Der Begriff "Nukleinsäuremolekül", wie hier verwendet, soll DNA-Moleküle (z.B. cDNA oder genomische DNA) und RNA-Moleküle (z.B. mRNA) sowie DNA- oder RNA-Analoga, die mittels Nukleotidanaloga erzeugt werden, umfassen. Dieser Begriff umfasst zudem die am 3'- und am 5'-Ende des kodierenden Genbereichs gelegene untranslatierte Sequenz: mindestens 500,

bevorzugt 200, besonders bevorzugt 100 Nukleotide der Sequenz stromaufwärts des 5'-Endes des kodierenden Bereichs und mindestens 100, bevorzugt 50, besonders bevorzugt 20 Nukleotide der Sequenz stromabwärts des 3'-Endes des kodierenden Genbereichs. Das Nukleinsäuremolekül kann einzelsträngig oder doppelsträngig sein, ist aber vorzugsweise doppelsträngige DNA. Ein "isoliertes" Nukleinsäuremolekül wird von anderen Nukleinsäuremolekülen abgetrennt, die in der natürlichen Quelle der Nukleinsäure vorliegen. Eine "isolierte" Nukleinsäure hat vorzugsweise keine Sequenzen, welche die Nukleinsäure in der genomischen DNA des Organismus, aus dem die Nukleinsäure stammt, natürlicherweise flankieren (z.B. Sequenzen, die sich an den 5'- und 3'-Enden der Nukleinsäure befinden). Bei verschiedenen Ausführungsformen kann das isolierte Desaturase-Nukleinsäuremolekül zum Beispiel weniger als etwa 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0,5 kb oder 0,1 kb an Nukleotidsequenzen enthalten, die natürlicherweise das Nukleinsäuremolekül in der genomischen DNA der Zelle, aus der die Nukleinsäure stammt (z.B. eine Physcomitrella patens-Zelle) flankieren. Ein "isoliertes" Nukleinsäuremolekül, wie ein cDNA-Molekül, kann überdies im wesentlichen frei von anderem zellulären Material oder Kulturmedium sein, wenn es durch rekombinante Techniken hergestellt wird, oder frei von chemischen Vorstufen oder anderen Chemikalien sein, wenn es chemisch synthetisiert wird.

Ein erfindungsgemäßes Nukleinsäuremolekül, z.B. ein Nukleinsäuremolekül mit einer Nukleotidsequenz der SEQ ID NO:1 oder eines Teils davon, kann unter Verwendung molekularbiologischer Standardtechniken und der hier bereitgestellten Sequenzinformation isoliert werden. Auch kann mithilfe von Vergleichsalgorithmen beispielsweise eine homologe Sequenz oder homologe, konservierte Sequenzbereiche auf DNA oder Aminosäureebene identifiziert werden. Beispielsweise kann aus einer Phaeodactylum tricornutum cDNA aus einer Phaeodactylum tricornutum-Bank isoliert werden, indem die vollständige SEQ ID NO:1, 3, 5 oder 11 oder ein Teil davon als Hybridisierungssonde sowie Standard-Hybridisierungstechniken (wie z.B. beschrieben in Sambrook et al., Molecular Cloning: A Laboratory Manual. 2. Aufl., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) verwendet werden. Überdies lässt sich ein Nukleinsäuremolekül, umfassend eine vollständige Sequenz der SEQ ID NO: 1, 3, 5 oder 11 oder einen Teil davon, durch Polymerasekettenreaktion isolieren, wobei Oligonukleotidprimer, die auf der Basis dieser Sequenz oder von Teilen davon, insbesondere Regionen um Motive aus Beispiel 5a erstellt werden oder Modifikationen ebensolcher in einzelnen definierten Aminosäuren, verwendet werden (z.B. kann ein Nukleinsäuremolekül, umfassend die

vollständigen Sequenz der SEQ ID NO:1, 3, 5 oder 11 oder einen Teil davon, durch Polymerasekettenreaktion unter Verwendung von Oligonukleotidprimern isoliert werden, die auf der Basis dieser gleichen Sequenz der SEQ ID NO: 1, 3, 5 oder 11 erstellt worden sind). Zum Beispiel lässt sich mRNA aus Zellen isolieren (z.B. durch das Guanidiniumthiocyanat-Extraktionsverfahren von Chirgwin et al. (1979) Biochemistry 18:5294-5299) und cDNA mittels Reverser Transkriptase (z.B. Moloney-MLV-Reverse-Transkriptase, erhältlich von Gibco/BRL, Bethesda, MD, oder AMV-Reverse-Transkriptase, erhältlich von Seikagaku America, Inc., St.Petersburg, FL) herstellen. Synthetische Oligonukleotidprimer zur Amplifizierung mittels Polymerasekettenreaktion lassen sich auf der Basis einer der in SEQ ID NO: 1, 3, 5 oder 11 sowie der in Figur 5a gezeigten Sequenzen oder mithilfe der in SEQ ID NO: 2, 4, 6 oder 12 dargestellten Aminosäuresequenzen erstellen. Eine erfindungsgemäße Nukleinsäure kann unter Verwendung von cDNA oder alternativ von genomischer DNA als Matrize und geeigneten Oligonukleotidprimern gemäß Standard-PCR-Amplifikationstechniken amplifiziert werden. Die so amplifizierte Nukleinsäure kann in einen geeigneten Vektor kloniert werden und mittels DNA-Sequenzanalyse charakterisiert werden. Oligonukleotide, die einer Desaturase-Nukleotidsequenz entsprechen, können durch Standard-Syntheseverfahren, beispielsweise mit einem automatischen DNA-Synthesegerät, hergestellt werden.

Die in SEQ ID NO: 1,3, 5 oder 11 gezeigte cDNA umfasst Sequenzen, die Desaturasen kodieren, (d.h. den "kodierenden Bereich") sowie 5'-untranslatierte Sequenzen und 3'-untranslatierte Sequenzen. Alternativ kann das Nukleinsäuremolekül nur den kodierenden Bereich einer der Sequenzen in SEQ ID NO: 1, 3, 5 oder 11 umfassen oder kann ganze genomische Fragmente, die aus genomischer DNA isoliert sind, enthalten.

Bei einer weiteren bevorzugten Ausführungsform umfasst ein erfindungsgemäßes isoliertes Nukleinsäuremolekül ein Nukleinsäuremolekül, das ein Komplement einer der in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Nukleotidsequenzen oder eines Teils davon ist. Ein Nukleinsäuremolekül, das zu einer der in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Nukleotidsequenzen komplementär ist, ist dann ausreichend komplementär, wenn es mit einer der in SEQ ID NO: 1, 3, 5 oder 11 angegebenen Sequenzen hybridisieren kann, wodurch ein stabiler Duplex entsteht.

Homologe der neuen Desaturase-Nukleinsäuresequenzen mit der Sequenz SEQ ID NO: 1, 3, 5 oder 11 bedeutet beispielsweise allelische Varianten mit mindestens etwa 50 bis 60 %, vorzugsweise mindestens etwa 60 bis 70 %, stärker bevorzugt mindestens

etwa 70 bis 80 %, 80 bis 90 % oder 90 bis 95 % und noch stärker bevorzugt mindestens etwa 95 %, 96 %, 97 %, 98 %, 99 % oder mehr Homologie zu einer in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Nukleotidsequenzen oder ihren Homologen, Derivaten oder Analoga oder Teilen davon. Bei einer weiteren bevorzugten Ausführungsform umfasst ein isoliertes erfindungsgemäßes Nukleinsäuremolekül eine Nukleotidsequenz, die an eine der in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Nukleotidsequenzen oder einen Teil davon hybridisiert, z.B. unter stringenten Bedingungen hybridisiert. Allelische Varianten umfassen insbesondere funktionelle Varianten, die sich durch Deletion, Insertion oder Substitution von Nukleotiden aus/in der in SEQ ID NO: 1, 3, 5 oder 11 dargestellten Sequenz erhalten lassen, wobei aber die Absicht ist, dass die Enzymaktivität der davon herrührenden synthetisierten Proteine für die Insertion eines oder mehrerer Gene vorteilhafterweise beibehalten wird. Proteine, die noch die enzymatische Aktivität der Desaturase besitzen, das heißt deren Aktivität im wesentlichen nicht reduziert ist, bedeutet Proteine mit mindestens 10 %, vorzugsweise 20 %, besonders bevorzugt 30 %, ganz besonders bevorzugt 40 % der ursprünglichen Enzymaktivität, verglichen mit dem durch SEQ ID NO: 2, 4, 6 oder 12 kodierten Protein.

Homologen der SEQ ID NO: 1, 3, 5 oder 11 bedeuten beispielsweise auch bakterielle, Pilz- und Pflanzenhomologen, verkürzte Sequenzen, einzelsträngige DNA oder RNA der kodierenden und nicht-kodierenden DNA-Sequenz.

Homologen der SEQ ID NO: 1, 3, 5 oder 11 bedeutet auch Derivate, wie beispielsweise Promotorvarianten. Die Promotoren stromaufwärts der angegebenen Nukleotidsequenzen können durch einen oder mehrere Nukleotidaustausche, durch Insertion(en) und/oder Deletion(en) modifiziert werden, ohne dass jedoch die Funktionalität oder Aktivität der Promotoren gestört wird. Es ist weiterhin möglich, dass die Aktivität der Promotoren durch Modifikation ihrer Sequenz erhöht ist oder dass sie vollständig durch aktivere Promotoren, sogar aus heterologen Organismen, ersetzt werden.

Überdies kann das erfindungsgemäße Nukleinsäuremolekül nur einen Teil des kodierenden Bereichs einer der Sequenzen in SEQ ID NO: 1, 3, 5 oder 11 umfassen, zum Beispiel ein Fragment, das als Sonde oder Primer verwendet werden kann, oder ein Fragment, welches einen biologisch aktiven Abschnitt einer Desaturase kodiert. Die aus der Klonierung des Desaturase-Gens von Phaeodactylum tricornutum ermittelten Nukleotidsequenzen ermöglichen die Erzeugung von Sonden und Primern, die zur Identifizierung und/oder Klonierung von Desaturase-Homologen in anderen

Zelltypen und Organismen sowie Desaturase-Homologen aus anderen Mikroalgen oder verwandten Arten gestaltet sind. Die Sonde/der Primer umfasst gewöhnlich im wesentlichen gereinigtes Oligonukleotid. Das Oligonukleotid umfasst gewöhnlich einen Nukleotidsequenzbereich, der unter stringenten Bedingungen an mindestens etwa 12, vorzugsweise etwa 16, stärker bevorzugt etwa 25, 40, 50 oder 75 aufeinanderfolgende Nukleotide eines Sense-Stranges einer der in SEQ ID NO: 1, 3, 5 oder 11 angegebenen Sequenzen, eines Antisense-Stranges einer der in SEQ ID NO: 1, 3, 5 oder 11 angegebenen Sequenzen oder seiner Homologen, Derivate oder Analoga oder natürlich vorkommender Mutanten davon hybridisiert. Primer auf der Basis einer Nukleotidsequenz der SEQ ID NO: 1, 3, 5 oder 11 können in PCR-Reaktionen zur Klonierung von Desaturase-Homologen verwendet werden. Sonden auf der Basis der Desaturase-Nukleotidsequenzen können zum Nachweis von Transkripten oder genomischen Sequenzen, die das gleiche oder homologe Proteine kodieren, verwendet werden. Bei bevorzugten Ausführungsformen umfasst die Sonde zudem eine daran gebundene Markierungsgruppe, z.B. ein Radioisotop, eine fluoreszierende Verbindung, ein Enzym oder einen Enzym-Cofaktor. Diese Sonden können als Teil eines Test-Kits für genomische Marker zur Identifizierung von Zellen, die eine Desaturase misexprimieren, beispielsweise durch Messen einer Menge einer Desaturase-kodierenden Nukleinsäure in einer Zellenprobe, z.B. Messen der Desaturase-mRNA-Spiegel, oder zur Bestimmung, ob ein genomisches Desaturase-Gen mutiert oder deletiert ist, verwendet werden.

Bei einer Ausführungsform kodiert das erfindungsgemäße Nukleinsäuremolekül ein Protein oder einen Teil davon, das/der eine Aminosäuresequenz umfasst, die ausreichend homolog zu einer Aminosäuresequenz der SEQ ID NO: 2, 4, 6 oder 12 ist, dass das Protein oder der Teil davon die Fähigkeit, am Stoffwechsel von zum Aufbau von Zellmembranen in Mikroorganismen oder Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilzunehmen, beibehält. Wie hier verwendet, betrifft der Begriff "ausreichend homolog" Proteine oder Teile davon, deren Aminosäuresequenzen eine minimale Anzahl identischer oder äquivalenter Aminosäurereste (z.B. einen Aminosäurerest mit einer ähnlichen Seitenkette, wie ein Aminosäurerest in einer der Sequenzen der SEQ ID NO:2) zu einer Aminosäuresequenz der SEQ ID NO: 2 aufweisen, so dass das Protein oder der Teil davon am Stoffwechsel von zum Aufbau von Zellmembranen in Mikroorganismen oder Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen kann. Proteinbestandteile dieser Stoffwechselwege für Membrankomponenten oder Membrantransportsysteme können, wie hier beschrieben, eine Rolle bei der Produktion und Sekretion einer oder mehrerer

Feinchemikalien spielen. Beispiele für diese Aktivitäten sind hier ebenfalls beschrieben. Somit trägt die "Funktion einer Desaturase" entweder direkt oder indirekt zur Ausbeute, Produktion und/oder Effizienz der Produktion einer oder mehrerer Feinchemikalien bei. Beispiele für Desaturase-Substratspezifitäten der katalytischen Aktivität sind in Tabelle 5 und 6 angegeben.

Bei einer weiteren Ausführungsform kodieren Derivate des erfindungsgemäßen Nukleinsäuremoleküls Proteine mit mindestens etwa 50 bis 60 %, vorzugsweise mindestens etwa 60 bis 70 % und stärker bevorzugt mindestens etwa 70 bis 80 %, 80 bis 90 %, 90 bis 95 % und am stärksten bevorzugt mindestens etwa 96 %, 97 %, 98 %, 99 % oder mehr Homologie zu einer vollständigen Aminosäuresequenz der SEQ ID NO:2. Die Homologie der Aminosäuresequenz kann über den gesamten Sequenzbereich mit dem Programm PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5, 1989:151-153) oder BESTFIT oder GAP bestimmt (Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919.)

Teile von Proteinen, die von den erfindungsgemäßen Desaturase-Nukleinsäuremolekülen kodiert werden, sind vorzugsweise biologisch aktive Teile einer der Desaturasen. Wie hier verwendet, soll der Begriff "biologisch aktiver Teil einer Desaturase", einen Abschnitt, z.B. eine Domäne/ein Motiv, einer Desaturase umfassen, der am Stoffwechsel von zum Aufbau von Zellmembranen in Mikroorganismen oder Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen kann oder eine in Tabelle 5 und 6 angegebene Aktivität aufweist. Zur Bestimmung, ob eine Desaturase oder ein biologisch aktiver Teil davon am Stoffwechsel von zum Aufbau von Zellmembranen in Mikroorganismen oder Pflanzen notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen kann, kann ein Test der enzymatischen Aktivität durchgeführt werden. Diese Testverfahren, wie eingehend in Beispiel 8 des Beispielteils beschrieben, sind dem Fachmann geläufig.

Zusätzliche Nukleinsäurefragmente, die biologisch aktive Abschnitte einer Desaturase kodieren, lassen sich durch Isolierung eines Teils einer der Sequenzen in SEQ ID NO: 1, 3, 5 oder 11, Exprimieren des kodierten Abschnitt der Desaturase oder des Peptids (z.B. durch rekombinante Expression in vitro) und Bestimmen der Aktivität des kodierten Teils der Desaturase oder des Peptids herstellen.

Die Erfindung umfasst zudem Nukleinsäuremoleküle, die sich von einer der in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Nukleotidsequenzen (und Teilen davon) aufgrund des degenerierten genetischen Codes unterscheiden und somit die gleiche Desaturase kodieren wie diejenige, die von den in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Nukleotidsequenzen kodiert wird. Bei einer anderen Ausführungsform hat ein erfindungsgemäßes isoliertes Nukleinsäuremolekül eine Nukleotidsequenz, die für ein Protein mit einer in SEQ ID NO: 2, 4, 6 oder 12 gezeigten Aminosäuresequenz kodiert. Bei einer weiteren Ausführungsform kodiert das erfindungsgemäße Nukleinsäuremolekül ein Vollängen-Desaturase-Protein, das zu einer Aminosäuresequenz der SEQ ID NO: 2,4, 6 oder 12 (die von einem in SEQ ID NO: 1, 3, 5 oder 11 gezeigten offenen Leseraster kodiert wird) im wesentlichen homolog ist und durch gängige Methoden identifizierbar und isolierbar ist.

Zusätzlich zu den in SEQ ID NO: 1, 3, 5 oder 11 gezeigten Desaturase-Nukleotidsequenzen erkennt der Fachmann, dass DNA-Sequenzpolymorphismen, die zu Änderungen in den Aminosäuresequenzen der Desaturasen führen, innerhalb einer Population (z.B. der Phaeodactylum tricornutum-Population) existieren können. Diese genetischen Polymorphismen im Desaturase-Gen können zwischen Individuen innerhalb einer Population aufgrund von natürlicher Variation existieren. Wie hier verwendet, bedeuten die Begriffe "Gen" und "rekombinantes Gen" Nukleinsäuremoleküle mit einem offenen Leserahmen, der eine Desaturase, vorzugsweise eine Phaeodactylum tricornutum -Desaturase, kodiert. Diese natürlichen Varianten bewirken üblicherweise eine Varianz von 1 bis 5 % in der Nukleotidsequenz des Desaturase-Gens. Sämtliche und alle dieser Nukleotidvariationen und daraus resultierende Aminosäurepolymorphismen in der Desaturase, die das Ergebnis natürlicher Variation sind und die funktionelle Aktivität von Desaturasen nicht verändern, sollen im Umfang der Erfindung enthalten sein.

Nukleinsäuremoleküle, die den natürlichen Varianten entsprechen, und nicht-Phaeodactylum tricornutum-Homologen, -Derivate und -Analoga der Phaeodactylum tricornutum-cDNA können auf der Grundlage ihrer Homologie zu der hier offenbarten Phaeodactylum tricornutum-Desaturase-Nukleinsäure unter Verwendung der Phaeodactylum tricornutum-cDNA oder eines Teils davon als Hybridisierungssonde gemäß Standard-Hybridisierungstechniken unter stringenten Hybridisierungsbedingungen isoliert werden. Bei einer anderen Ausführungsform ist ein erfindungsgemäßes isoliertes Nukleinsäuremolekül mindestens 15 Nukleotide lang und hybridisiert unter stringenten Bedingungen mit dem Nukleinsäuremolekül, das eine Nukleotidsequenz der SEQ ID NO:1, 3, 5 oder 11 umfasst.

Bei anderen Ausführungsformen ist die Nukleinsäure mindestens 25, 50, 100, 250 oder mehr Nukleotide lang. Der Begriff "hybridisiert unter stringenten Bedingungen", wie hier verwendet, soll Hybridisierungs- und Waschbedingungen beschreiben, unter denen Nukleotidsequenzen, die mindestens 60 % homolog zueinander sind, gewöhnlich aneinander hybridisiert bleiben. Die Bedingungen sind vorzugsweise derart, dass Sequenzen, die mindestens etwa 65 %, stärker bevorzugt mindestens etwa 70 % und noch stärker bevorzugt mindestens etwa 75 % oder stärker zueinander homolog sind, gewöhnlich aneinander hybridisiert bleiben. Diese stringenten Bedingungen sind dem Fachmann bekannt und lassen sich in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6., finden. Ein bevorzugtes, nicht einschränkendes Beispiel für stringente Hybridisierungsbedingungen sind Hybridisierungen in 6 x Natriumchlorid/Natriumcitrat (sodium chloride/ sodiumcitrate = SSC) bei etwa 45°C, gefolgt von einem oder mehreren Waschschritten in 0,2 x SSC, 0,1 % SDS bei 50 bis 65°C. Dem Fachmann ist bekannt, dass diese Hybridisierungsbedingungen sich je nach dem Typ der Nukleinsäure und, wenn beispielsweise organische Lösungsmittel vorliegen, hinsichtlich der Temperatur und der Konzentration des Puffers unterscheiden. Die Temperatur unterscheidet sich beispielsweise unter "Standard-Hybridisierungsbedingungen" je nach dem Typ der Nukleinsäure zwischen 42°C und 58°C in wässrigem Puffer mit einer Konzentration von 0,1 bis 5 x SSC (pH 7,2). Falls organisches Lösungsmittel im obengenannten Puffer vorliegt, zum Beispiel 50 % Formamid, ist die Temperatur unter Standardbedingungen etwa 42°C. Vorzugsweise sind die Hybridisierungsbedingungen für DNA:DNA-Hybride zum Beispiel 0,1 x SSC und 20°C bis 45°C, vorzugsweise zwischen 30°C und 45°C. Vorzugsweise sind die Hybridisierungsbedingungen für DNA:RNA-Hybride zum Beispiel 0,1 x SSC und 30°C bis 55°C, vorzugsweise zwischen 45°C und 55°C. Die vorstehend genannten Hybridisierungstemperaturen sind beispielsweise für eine Nukleinsäure mit etwa 100 bp (= Basenpaare) Länge und einem G + C-Gehalt von 50 % in Abwesenheit von Formamid bestimmt. Der Fachmann weiß, wie die erforderlichen Hybridisierungsbedingungen anhand von Lehrbüchern, wie dem vorstehend erwähnten oder aus den folgenden Lehrbüchern Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames und Higgins (Hrsgb.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Hrsgb.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford, bestimmt werden können.

Vorzugsweise entspricht ein erfindungsgemäßes isoliertes Nukleinsäuremolekül, das unter stringenten Bedingungen an eine Sequenz der SEQ ID NO:1, 3, 5 oder 11 hybridisiert, einem natürlich vor-

kommenden Nukleinsäuremolekül. Wie hier verwendet, betrifft ein "natürlich vorkommendes" Nukleinsäuremolekül ein RNA- oder DNA-Molekül mit einer Nukleotidsequenz, die in der Natur vorkommt (z.B. ein natürliches Protein kodiert). Bei einer Ausführungsform kodiert die Nukleinsäure eine natürliche vorkommendes Phyaeodactylum tricornutum-Desaturase.

Zusätzlich zu natürlich vorkommenden Varianten der Desaturasesequenz, die in der Population existieren können, erkennt der Fachmann ferner, dass auch Änderungen durch Mutation in eine Nukleotidsequenz der SEQ ID NO: 1, 3, 5 oder 11 eingebracht werden können, was zu Änderungen der Aminosäuresequenz der kodierten Desaturase führt, ohne dass die Funktionsfähigkeit des Desaturaseproteins beeinträchtigt wird. Beispielsweise lassen sich Nukleotidsubtitutionen, die an "nicht-essentiellen" Aminosäureresten zu Aminosäuresubstitutionen führen, in einer Sequenz der SEQ ID NO: 2, 4, 6 oder 12 herstellen. Ein "nicht-essentieller" Aminosäurerest ist ein Rest, der sich in einer Wildtyp-Desaturasequenz einer der Desaturasen (SEQ ID NO: 2, 4, 6 oder 12) verändern lässt, ohne dass die Aktivität der Desaturase verändert das heißt wesentlich reduziert wird, wohingegen ein "essentieller" Aminosäurerest für die Desaturaseaktivität erforderlich ist. Andere Aminosäurereste (z.B. diejenigen, die in der Domäne mit Desaturaseaktivität nicht konserviert oder lediglich semikonserviert sind) können jedoch für die Aktivität nicht essentiell sein und lassen sich somit verändern, ohne dass die Desaturaseaktivität verändert wird.

Folglich betrifft ein weiterer Aspekt der Erfindung Nukleinsäuremoleküle, die Desaturasen kodieren, die veränderte Aminosäurereste enthalten, die für die Desaturaseaktivität nicht essentiell sind. Diese Desaturasen unterscheiden sich in der Aminosäuresequenz von einer Sequenz in SEQ ID NO: 2, 4, 6 oder 12 und behalten dennoch zumindest eine der hier beschriebenen Desaturaseaktivitäten. Das isolierte Nukleinsäuremolekül umfasst bei einer Ausführungsform eine Nukleotidsequenz, die ein Protein kodiert, wobei das Protein eine Aminosäuresequenz mit mindestens etwa 50 % Homologie zu einer Aminosäuresequenz der SEQ ID NO: 2, 4, 6 oder 12 umfasst und am Stoffwechsel von zum Aufbau von Zellmembranen in Phaeodactylum tricornutum notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen kann. Das von dem Nukleinsäuremolekül kodierte Protein ist vorzugsweise mindestens etwa 50 bis 60 % homolog zu einer der Sequenzen in SEQ ID NO:2, 4, 6 oder 12 stärker bevorzugt mindestens etwa 60 bis 70 % homolog zu einer der Sequenzen in SEQ ID NO:2, 4, 6 oder 12 noch stärker bevorzugt mindestens etwa 70 bis 80 %, 80 bis 90 %, 90 bis 95 % homolog zu einer

der Sequenzen in SEQ ID NO: 2, 4, 6 oder 12 und am stärksten bevorzugt mindestens etwa 96 %, 97 %, 98 % oder 99 % homolog zu einer der Sequenzen in SEQ ID NO: 2, 4, 6 oder 12.

Zur Bestimmung der prozentualen Homologie von zwei Aminosäuresequenzen (z.B. einer der Sequenzen der SEQ ID NO: 2, 4, 6 oder 12 und einer mutierten Form davon) oder von zwei Nukleinsäuren werden die Sequenzen zum Zweck des optimalen Vergleichs untereinander geschrieben (z.B. können Lücken in die Sequenz eines Proteins oder einer Nukleinsäure eingefügt werden, um ein optimales Alignment mit dem anderen Protein oder der anderen Nukleinsäure zu erzeugen). Die Aminosäurereste oder Nukleotide an den entsprechenden Aminosäurepositionen oder Nukleotidpositionen werden dann verglichen. Wenn eine Position in einer Sequenz (z.B. einer der Sequenzen der SEQ ID NO: 2, 4, 6 oder 12) durch den gleichen Aminosäurerest oder das gleiche Nukleotid wie die entsprechende Stelle in der anderen Sequenz (z.B. einer mutierten Form der aus SEQ ID NO: 2, 4, 6 oder 12 ausgewählten Sequenz) belegt wird, dann sind die Moleküle an dieser Position homolog (d.h. Aminosäure- oder Nukleinsäure-"Homologie", wie hier verwendet, entspricht Aminosäure- oder Nukleinsäure-"Identität"). Die prozentuale Homologie zwischen den beiden Sequenzen ist eine Funktion der Anzahl an identischen Positionen, die den Sequenzen gemeinsam sind (d.h. % Homologie = Anzahl der identischen Positionen/Gesamtanzahl der Positionen x 100). Die Begriffe Homologie und Identität sind damit als Synonym anzusehen.

Ein isoliertes Nukleinsäuremolekül, das eine Desaturase kodiert, die zu einer Proteinsequenz der SEQ ID NO: 2, 4, 6 oder 12 homolog ist, kann durch Einbringen einer oder mehrerer Nukleotidsubstitutionen, -additionen oder -deletionen in eine Nukleotidsequenz der SEQ ID NO: 1, 3, 5 oder 11 erzeugt werden, so dass eine oder mehrere Aminosäuresubstitutionen, -additionen oder -deletionen in das kodierte Protein eingebracht werden. Mutationen können in eine der Sequenzen der SEQ ID NO: 1, 3, 5 oder 11 durch Standardtechniken, wie stellenspezifische Mutagenese und PCR-vermittelte Mutagenese, eingebracht werden. Vorzugsweise werden konservative Aminosäuresubstitutionen an einer oder mehreren der vorhergesagten nicht-essentiellen Aminosäureresten hergestellt. Bei einer "konservativen Aminosäuresubstitution" wird der Aminosäurerest gegen einen Aminosäurerest mit einer ähnlichen Seitenkette ausgetauscht. Im Fachgebiet sind Familien von Aminosäureresten mit ähnlichen Seitenketten definiert worden. Diese Familien umfassen Aminosäuren mit basischen Seitenketten (z.B. Lysin, Arginin, Histidin), sauren Seitenketten (z.B. Asparaginsäure, Glutaminsäure), ungeladenen

polaren Seitenketten (z.B. Glycin, Asparagin, Glutamin, Serin, Threonin, Tyrosin, Cystein), unpolaren Seitenketten, (z.B. Alanin, Valin, Leucin, Isoleucin, Prolin, Phenylalanin, Methionin, Tryptophan), beta-verzweigten Seitenketten (z.B. Threonin, Valin, Isoleucin) und aromatischen Seitenketten (z.B. Tyrosin, Phenylalanin, Tryptophan, Histidin). Ein vorhergesagter nicht-essentieller Aminosäurerest in einer Desaturase wird somit vorzugsweise durch einen anderen Aminosäurerest aus der gleichen Seitenkettenfamilie ausgetauscht. Alternativ können bei einer anderen Ausführungsform die Mutationen zufallsgemäß über die gesamte oder einen Teil der Desaturase-kodierenden Sequenz eingebracht werden, z.B. durch Sättigungsmutagenese, und die resultierenden Mutanten können nach der hier beschriebenen Desaturase-Aktivität durchmustert werden, um Mutanten zu identifizieren, die Desaturaseaktivität beibehalten. Nach der Mutagenese einer der Sequenzen der SEQ ID NO: 1, 3, 5 oder 11 kann das kodierte Protein rekombinant exprimiert werden, und die Aktivität des Proteins kann z.B. unter Verwendung der hier beschriebenen Tests (siehe Beispielteil) bestimmt werden.

Zusätzlich zu den Nukleinsäuremolekülen, welche die vorstehend beschriebenen Desaturasen kodieren, betrifft ein weiterer Aspekt der Erfindung isolierte Nukleinsäuremoleküle, die "Antisense" zu den erfindungsgemäßen Nukleinsäuresequenzen sind. Eine "Antisense"-Nukleinsäure umfasst eine Nukleotidsequenz, die zu einer "Sense"-Nukleinsäure, welche ein Protein kodiert, komplementär ist, z.B. komplementär zum kodierenden Strang eines doppelsträngigen cDNA-Moleküls oder komplementär zu einer mRNA-Sequenz. Eine Antisense-Nukleinsäure kann folglich über Wasserstoffbrückenbindungen an eine Sense-Nukleinsäure binden. Die Antisense-Nukleinsäure kann zu einem gesamten Desaturase-kodierenden Strang oder nur zu einem Teil davon komplementär sein. Bei einer Ausführungsform ist ein Antisense-Nukleinsäuremolekül "Antisense" zu einem "kodierenden Bereich" des kodierenden Strangs einer Nukleotidsequenz, die eine Desaturase kodiert. Der Begriff "kodierender Bereich" betrifft den Bereich der Nukleotidsequenz, der Codons umfasst, die in Aminosäurereste translatiert werden (z.B. den gesamten kodierenden Bereich, der mit dem Stopcodon beginnt und endet, d.h. dem letzten Codon vor dem Stopcodon). Bei einer weiteren Ausführungsform ist das Antisense-Nukleinsäuremolekül "Antisense" zu einem "nicht-kodierenden Bereich" des kodierenden Strangs einer Nukleotidsequenz, die Desaturase kodiert. Der Begriff "nicht-kodierender Bereich" betrifft 5'- und 3'-Sequenzen, die den kodierenden Bereich flankieren und nicht in Aminosäuren translatiert werden (d.h. die man auch als 5'- und 3'-untranslatierte Bereiche bezeichnet).

Unter Voraussetzung der hier offenbarten Desaturase-kodierenden Sequenzen des kodierenden Stranges (z.B. die in SEQ ID NO: 1, 3, 5 oder 11 dargestellten Sequenzen) können erfindungsgemäße Antisense-Nukleinsäuren gemäß den Regeln der Watson-Crick-Basenpaarung gestaltet werden. Das Antisense-Nukleinsäuremolekül kann komplementär zum gesamten kodierenden Bereich von Desaturase-mRNA sein, ist aber stärker bevorzugt ein Oligonukleotid, das nur zu einem Teil des kodierenden oder nicht-kodierenden Bereichs von Desaturase-mRNA "Antisense" ist. Das Antisense-Oligonukleotid kann z.B. zu dem Bereich, der die Translationsstartstelle von Desaturase-mRNA umgibt, komplementär sein. Ein Antisense-Oligonukleotid kann z.B. etwa 5, 10, 15, 20, 25, 30, 35, 40, 45 oder 50 und mehr Nukleotide lang sein. Eine erfindungsgemäße Antisense-Nukleinsäure kann unter Verwendung chemischer Synthese und enzymatischer Ligationsreaktionen mittels im Fachgebiet bekannter Verfahren konstruiert werden. Eine Antisense-Nukleinsäure (z.B. ein Antisense-Oligonukleotid) kann z.B. chemisch synthetisiert werden, wobei natürlich vorkommende Nukleotide oder verschiedentlich modifizierte Nukleotide verwendet werden, die so gestaltet sind, dass sie die biologische Stabilität der Moleküle erhöhen oder die physikalische Stabilität des zwischen der Antisense- und der Sense-Nukleinsäure gebildeten Duplexes erhöhen, beispielsweise können Phosphorthioat-Derivate und acridinsubstituierte Nukleotide verwendet werden. Beispiele für modifizierte Nukleotide, die zur Erzeugung der Antisense-Nukleinsäure verwendet werden können, sind u.a. 5-Fluoruracil, 5-Bromuracil, 5-Chloruracil, 5-Ioduracil, Hypoxanthin, Xanthin, 4-Acetylcytosin, 5-(Carboxyhydroxylmethyl)uracil, 5-Carboxymethylaminomethyl-2-thiouridin, 5-Carboxymethylaminomethyluracil, Dihydrouracil, Beta-D-Galactosylqueosin, Inosin, N6-Isopentenyladenin, 1-Methylguanin, 1-Methylinosin, 2,2-Dimethylguanin, 2-Methyladenin, 2-Methylguanin, 3-Methylcytosin, 5-Methylcytosin, N6-Adenin, 7-Methylguanin, 5-Methylaminomethyluracil, 5-Methoxyaminomethyl-2-thiouracil, Beta-D-Mannosylqueosin, 5'-Methoxycarboxymethyluracil, 5-Methoxyuracil, 2-Methylthio-N6-isopentyladenin, Uracil-5-oxyessigsäure (v), Wybutoxosin, Desaturaseudouracil, Queosin, 2-Thiocytosin, 5-Methyl-2-thiouracil, 2-Thiouracil, 4-Thiouracil, 5-Methyluracil, Uracil-5-oxyessigsäuremethylester, Uracil-5-oxyessigsäure (v), 5-Methyl-2-thiouracil, 3-(3-Amino-3-N-2-carboxypropyl)uracil, (acp3)w und 2,6-Diaminopurin. Die Antisense-Nukleinsäure kann alternativ biologisch unter Verwendung eines Expressionsvektors hergestellt werden, in den eine Nukleinsäure in Antisense-Richtung subkloniert worden ist (d.h. RNA, die von der eingebrachten Nukleinsäure transkribiert wird, ist zu einer Zielnukleinsäure von Interesse in Antisense-Richtung orientiert, was im nachstehenden Unterabschnitt weiter beschrieben ist).

Die erfindungsgemäßen Antisense-Nukleinsäuremoleküle werden üblicherweise an eine Zelle verabreicht oder in situ erzeugt, so dass sie mit der zellulären mRNA und/oder der genomischen DNA, die eine Desaturase kodiert, hybridisieren oder daran binden, um dadurch die Expression des Proteins, z.B. durch Hemmung der Transkription und/oder Translation, zu hemmen. Die Hybridisierung kann durch herkömmliche Nukleotidkomplementarität unter Bildung eines stabilen Duplexes oder z.B. im Fall eines Antisense-Nukleinsäuremoleküls, das DNA-Duplices bindet, durch spezifische Wechselwirkungen in der großen Furche der Doppelhelix erfolgen. Das Antisense-Molekül kann so modifiziert sein, dass es spezifisch an einen Rezeptor oder an ein auf einer ausgewählten Zelloberfläche exprimiertes Antigen bindet, z.B. durch Binden des Antisense-Nukleinsäuremoleküls an ein Peptid oder einen Antikörper, das/der an einen Zelloberflächenrezeptor oder ein Antigen bindet. Das Antisense-Nukleinsäuremolekül kann auch unter Verwendung der hier beschriebenen Vektoren den Zellen zugeführt werden. Zur Erzielung ausreichender intrazellulärer Konzentrationen der Antisense-Moleküle sind Vektorkonstrukte, in denen sich das Antisense-Nukleinsäuremolekül unter der Kontrolle eines starken prokaryotischen, viralen oder eukaryotischen, einschließlich pflanzlichen, Promotors befindet, bevorzugt.

Bei einer weiteren Ausführungsform ist das erfindungsgemäße Antisense-Nukleinsäuremolekül ein α-anomeres Nukleinsäuremolekül. Ein α-anomeres Nukleinsäuremolekül bildet spezifische doppelsträngige Hybride mit komplementärer RNA, wobei die Stränge im Gegensatz zu gewöhnlichen β-Einheiten parallel zueinander verlaufen. (Gaultier et al. (1987) Nucleic Acids Res. 15:6625-6641). Das Antisense-Nukleinsäuremolekül kann zudem ein 2'-o-Methylribonukleotid (Inoue et al. (1987) Nucleic Acids Res. 15:6131-6148) oder ein chimäres RNA-DNA-Analogon (Inoue et al. (1987) FEBS Lett. 215:327-330) umfassen.

Bei einer weiteren Ausführungsform ist eine erfindungsgemäße Antisense-Nukleinsäure ein Ribozym. Ribozyme sind katalytische RNA-Moleküle mit Ribonukleaseaktivität, die eine einzelsträngige Nukleinsäure, wie eine mRNA, spalten können, zu der sie einen komplementären Bereich haben. Somit können Ribozyme (z.B. Hammerhead-Ribozyme (beschrieben in Haselhoff und Gerlach (1988) Nature 334:585-591)) zur katalytischen Spaltung von Desaturase-mRNA-Transkripten verwendet werden, um dadurch die Translation von Desaturase-mRNA zu hemmen. Ein Ribozym mit Spezifität für eine Desaturase-kodierende Nukleinsäure kann auf der Basis der Nukleotidsequenz einer der in SEQ ID NO: 1, 3, 5 oder 11 offenbarten Desaturase-cDNA (d.h. oder auf der Basis einer gemäß den in dieser Erfindung gelehrten Verfahren zu isolierenden heterologen

Sequenz gestaltet werden. Beispielsweise kann ein Derivat einer Tetrahymena-L-19-IVS-RNA konstruiert werden, wobei die Nukleotidsequenz der aktiven Stelle komplementär zu der Nukleotidsequenz ist, die in einer Desaturase-kodierenden mRNA gespalten werden soll. Siehe z.B. Cech et al., US-Patent Nr. 4,987,071 und Cech et al., US-Patent Nr. 5,116,742. Alternativ kann Desaturase-mRNA zur Selektion einer katalytischen RNA mit einer spezifischen Ribonukleaseaktivität aus einem Pool von RNA-Molekülen verwendet werden. Siehe z.B. Bartel, D., und Szostak, J.W. (1993) Science 261:1411-1418.

Alternativ lässt sich die Desaturase-Gen-Expression hemmen, indem Nukleotidsequenzen, die komplementär zum regulatorischen Bereich einer Desaturase-Nukleotidsequenz (z.B. einem Desaturase-Promotor und/oder -Enhancer) sind, so dirigiert werden, dass Dreifachhelix-Strukturen gebildet werden, welche die Transkription eines Desaturase-Gens in Zielzellen hemmen. Siehe allgemein Helene, C. (1991) Anticancer Drug Res. 6(6) 569-84; Helene, C., et al. (1992) Ann. N. Y. Acad. Sci. 660:27-36; und Maher. L.J. (1992) Bioassays 14(12):807-815.

B. Genkonstrukt (= Nukleinsäurekonstrukt, -fragment oder Expressionskassette)

Unter der erfindungsgemäßen Expressionskassette sind die in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 genannten Sequenzen, die sich als Ergebnis des genetischen Codes und/oder deren funktionellen oder nicht funktionellen Derivate zu verstehen, die mit einem oder mehreren Regulationssignalen vorteilhafterweise zur Erhöhung der Genexpression funktionell verknüpft wurden und welche vorteilhaft die Expression der codierenden Sequenz in der Wirtszelle steuern. Diese regulatorischen Sequenzen sollen die gezielte Expression der Gene und der Proteinexpression ermöglichen. Dies kann beispielsweise je nach Wirtsorganismus bedeuten, dass das Gen erst nach Induktion exprimiert und/oder überexprimiert wird, oder dass es sofort exprimiert und/oder überexprimiert wird. Beispielsweise handelt es sich bei diesen regulatorischen Sequenzen um Sequenzen an die Induktoren oder Repressoren binden und so die Expression der Nukleinsäure regulieren. Zusätzlich zu diesen neuen Regulationssequenzen oder anstelle dieser Sequenzen kann die natürliche Regulation dieser Sequenzen vor den eigentlichen Strukturgenen noch vorhanden sein und gegebenenfalls genetisch verändert worden sein, so dass die natürliche Regulation ausgeschaltet und die Expression der Gene erhöht wurde. Das Genkonstrukt kann aber auch einfacher aufgebaut sein, das heißt es wurden keine zusätzlichen Regulationssignale vor die Nukleinsäuresequenz oder

dessen Derivate inseriert und der natürliche Promotor mit seiner Regulation wurde nicht entfernt. Stattdessen wurde die natürliche Regulationssequenz so mutiert, dass keine Regulation mehr erfolgt und/oder die Genexpression gesteigert wird. Diese veränderten Promotoren können in Form von Teilsequenzen (= Promotor mit Teilen der erfindungsgemäßen Nukleinsäuresequenzen) auch allein vor das natürliche Gen zur Steigerung der Aktivität gebracht werden. Das Genkonstrukt kann außerdem vorteilhafterweise auch eine oder mehrere sogenannte "enhancer Sequenzen" funktionell verknüpft mit dem Promotor enthalten, die eine erhöhte Expression der Nukleinsäuresequenz ermöglichen. Auch am 3'-Ende der DNA-Sequenzen können zusätzliche vorteilhafte Sequenzen inseriert werden wie weitere regulatorische Elemente oder Terminatoren. Die Δ-5-Desaturase-/Δ-6-Desaturase und/oder Δ-12-Desaturasegene können in einer oder mehreren Kopien in der Expressionskassette (= Genkonstrukt) enthalten sein.

Die regulatorischen Sequenzen bzw. Faktoren können dabei wie oben beschrieben vorzugsweise die Genexpression der eingeführten Gene positiv beeinflussen und dadurch erhöhen. So kann eine Verstärkung der regulatorischen Elemente vorteilhafterweise auf der Transkriptionsebene erfolgen, indem starke Transkriptionssignale wie Promotoren und/oder "Enhancer" verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert wird.

Eine weitere Ausführungsform der Erfindung sind ein oder mehrere Genkonstrukte, die eine oder mehrere Sequenzen enthalten, die durch Seq ID NO: 1, 3, 5, 7, 9 oder 11 definiert sind und gem. SEQ ID NO: 2, 4, 6, 8, 10 oder 12 Polypeptide kodieren. Dabei stammen SEQ ID NO: 1, 3, 5, 7 und 11 von Desaturasen während SEQ ID NO: 9 für eine Elongase codiert. Desaturasen codierende Enzyme, die eine Doppelbindung in Δ-5-, Δ-6- oder Δ-12-Position einführen, wobei das Substrat ein, zwei, drei oder vier Doppelbindungen aufweisen. Die in SEQ ID NO: 9 dargestellte Sequenz codiert für eine Enzymaktivität, die eine Fettsäure um mindestens zwei Kohlenstoffatome verlängert sowie ihre Homologen, Derivate oder Analoga, die funktionsfähig mit einem oder mehreren Regulationssignalen, vorteilhafterweise zur Steigerung der Genexpression, verbunden sind. Beispiele für diese Regulationssequenzen sind Sequenzen, an die Induktoren oder Repressoren binden und so die Expression der Nukleinsäure regulieren. Zusätzlich zu diesen neuen Regulationssequenzen kann die natürliche Regulation dieser Sequenzen vor den eigentlichen Strukturgenen noch vorhanden sein und, wenn geeignet, genetisch modifiziert worden sein, so dass die natürliche Regulation ausgeschaltet worden ist und die Expression der Gene gesteigert worden ist.

Das Genkonstrukt kann jedoch auch eine einfachere Struktur haben, d.h. dass keine zusätzlichen Regulationssignale vor der Sequenz SEQ ID NO: 1, 3, 5 oder 11 oder ihren Homologen inseriert worden sind und der natürliche Promotor mit seiner Regulation nicht deletiert worden ist. Statt dessen ist die natürliche Regulationssequenz so mutiert worden, dass keine Regulation mehr stattfindet und die Genexpression verstärkt ist. Das Genkonstrukt kann außerdem vorteilhafterweise eine oder mehrere sogenannte Enhancer-Sequenzen, die funktionsfähig mit dem Promotor verbunden sind und die gesteigerte Expression der Nukleinsäuresequenz ermöglichen, umfassen. Es ist auch möglich, am 3'-Ende der DNA-Sequenzen zusätzlich vorteilhafte Sequenzen zu inserieren, beispielsweise weitere Regulationselemente oder Terminatoren. Die Desaturasegene und das Elongasegen können im Genkonstrukt in einer oder mehreren Kopien vorliegen. Sie können in einem Genkonstrukt oder mehreren Genkonstrukten vorliegen. Dieses Genkonstrukt oder die Genkonstrukte können zusammen im Wirtsorganismus exprimiert werden. Dabei kann das Genkonstrukt oder die Genkonstrukte in einem oder mehreren Vektoren inseriert sein und frei in der Zelle vorliegen oder aber im Genom inseriert sein. Es ist vorteilhaft für die Insertion weiterer Gene in Organismen, wenn weitere Gene im Genkonstrukt vorliegen.

Vorteilhafte Regulationssequenzen für das neue Verfahren liegen beispielsweise in Promotoren vor, wie dem cos-, tac-, trp-, tet-, trp-tet-, lpp-, lac-, lpp-lac-, $lacI^q$-, T7-, T5-, T3-, gal-, trc-, ara-, SP6-, λ-P_R- oder λ-P_L-Promotor und werden vorteilhafterweise in Gram-negativen Bakterien angewendet. Weitere vorteilhafte Regulationssequenzen liegen beispielsweise in den Gram-positiven Promotoren amy und SPO2, in den Hefe- oder Pilzpromotoren ADC1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH oder in den Pflanzenpromotoren CaMV/35S [Franck et al., Cell 21 (1980) 285-294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, nos oder im Ubiquitin- oder Phaseolin-Promotor vor. In diesem Zusammenhang vorteilhaft sind ebenfalls induzierbare Promotoren, wie die in EP-A-0 388 186 (Benzylsulfonamid-induzierbar), Plant J. 2, 1992:397-404 (Gatz et al., Tetracyclin-induzierbar), EP-A-0 335 528 (Abzisinsäure-induzierbar) oder WO 93/21334 (Ethanol- oder Cyclohexenol-induzierbar) beschriebenen Promotoren. Weitere geeignete Pflanzenpromotoren sind der Promotor von cytosolischer FBPase oder der ST-LSI-Promotor der Kartoffel (Stockhaus et al., EMBO J. 8, 1989, 2445), der Phosphoribosylpyrophosphatamidotransferase-Promotor aus Glycine max (Genbank-Zugangsnr. U87999) oder der in EP-A-0 249 676 beschriebene nodienspezifische Promotor. Besonders vorteilhafte Promotoren sind Promotoren, welche die Expression in Geweben ermöglichen, die an der Fettsäurebiosynthese beteiligt sind. Ganz

besonders vorteilhaft sind samenspezifische Promotoren, wie der ausführungsgemäße USP Promotor aber auch andere Promotoren wie der LeB4- (Baeumlein et al., Plant J., 1992, 2 (2) (2):233-239), DC3 (Thomas, Plant Cell 1996, 263:359-368), Phaseolin- oder Napin-Promotor. Weitere besonders vorteilhafte Promotoren sind samenspezifische Promotoren, die für monokotyle oder dikotyle Pflanzen verwendet werden können und in US 5,608,152 (Napin-Promotor aus Raps), WO 98/45461 (Oleosin-Promotor aus Arobidopsis), US 5,504,200 (Phaseolin-Promotor aus Phaseolus vulgaris), WO 91/13980 (Bce4-Promotor aus Brassica), von Baeumlein et al., Plant J., 1992, 2 (2):233-239 (LeB4-Promotor aus einer Leguminose) beschrieben sind, wobei sich diese Promotoren für Dikotyledonen eignen. Die folgenden Promotoren eignen sich beispielsweise für Monokotyledonen lpt-2- oder lpt-1-Promotor aus Gerste (WO 95/15389 und WO 95/23230), Hordein-Promotor aus Gerste und andere, in WO 99/16890 beschriebene geeignete Promotoren.

Es ist im Prinzip möglich, alle natürlichen Promotoren mit ihren Regulationssequenzen, wie die oben genannten, für das neue Verfahren zu verwenden. Es ist ebenfalls möglich und vorteilhaft, zusätzlich synthetische Promotoren zu verwenden.

Das Genkonstrukt kann, wie oben beschrieben, auch weitere Gene umfassen, die in die Organismen eingebracht werden sollen. Es ist möglich und vorteilhaft, in die Wirtsorganismen Regulationsgene, wie Gene für Induktoren, Repressoren oder Enzyme, welche durch ihre Enzymaktivität in die Regulation eines oder mehrerer Gene eines Biosynthesewegs eingreifen, einzubringen und darin zu exprimieren. Diese Gene können heterologen oder homologen Ursprungs sein. Weiterhin können vorteilhaft im Nukleinsäurekonstrukt bzw. Genkonstrukt weitere Biosynthesegene des Fettsäure- oder Lipidstoffwechsels enthalten sein oder aber diese Gene können auf einem weiteren oder mehreren weiteren Nukleinsäurekonstrukten liegen. Vorteilhaft werden als Biosynthesegene des Fettsäure- oder Lipidstoffwechsels ein Gen ausgewählt aus der Gruppe Acyl-CoA-Dehydrogenase(n), Acyl-ACP[= acyl carrier protein]-Desaturase(n), Acyl-ACP-Thioesterase(n), Fettsäure-Acyl-Transferase(n), Fettsäure-Synthase(n), Fettsäure-Hydroxylase(n), Acetyl-Coenzym A-Carboxylase(n), Acyl-Coenzym, A-Oxidase(n), Fettsäure-Desaturase(n), Fettsäure-Acetylenasen, Lipoxygenasen, Triacylglycerol-Lipasen, Allenoxid-Synthasen, Hydroperoxid-Lyasen oder Fettsäure-Elongase(n) oder deren Kombinationen verwendet.

Genkonstrukte umfassen vorteilhafterweise zur Expression der anderen vorliegenden Gene weitere 3'- und/oder 5'-terminale Regulationssequenzen zur Steigerung der Expression, die in Abhängigkeit vom gewählten Wirtsorganismus und dem Gen oder den Genen für die optimale Expression ausgewählt werden. Diese Regulationssequenzen sollen, wie oben erwähnt, die spezifische Expression der Gene und die Proteinexpression ermöglichen. Dies kann je nach dem Wirtsorganismus beispielsweise bedeuten, dass das Gen nur nach Induktion exprimiert oder überexprimiert wird oder dass es sofort exprimiert und/oder überexprimiert wird.

Die Regulationssequenzen oder -faktoren können außerdem vorzugsweise eine vorteilhafte Wirkung auf die Expression der eingebrachten Gene haben und diese somit steigern. Auf diese Weise ist es möglich, dass die Regulationselemente unter Verwendung starker Transkriptionssignale, wie Promotoren und/oder Enhancer, vorteilhafterweise auf Transkriptionsebene verstärkt werden. Es ist jedoch weiterhin auch möglich, die Translation zum Beispiel durch Verbesserung der mRNA-Stabilität zu verstärken.

C. Rekombinante Expressionsvektoren und Wirtszellen

Ein weiterer Aspekt der Erfindung betrifft Vektoren, vorzugsweise Expressionsvektoren, die eine Nukleinsäure enthalten, die eine Desaturase allein (oder einen Teil davon) oder ein unter Punkt b beschriebenes Nukleinsäurekonstrukt in dem die erfindungsgemäße Nukleinsäure allein oder in Kombination mit weiteren Biosynthesegenen des Fettsäure- oder Lipidstoffwechsels wie Desaturasen oder Elongasen enthalten ist. Wie hier verwendet, betrifft der Begriff "Vektor" ein Nukleinsäuremolekül, das eine andere Nukleinsäure transportieren kann, an welche es gebunden ist. Ein Vektortyp ist ein "Plasmid", was für eine zirkuläre doppelsträngige DNA-Schleife steht, in die zusätzlichen DNA-Segmente ligiert werden können. Ein weiterer Vektortyp ist ein viraler Vektor, wobei zusätzliche DNA-Segmente in das virale Genom ligiert werden können. Bestimmte Vektoren können in einer Wirtszelle, in die sie eingebracht worden sind, autonom replizieren (z.B. Bakterienvektoren mit bakteriellem Replikationsursprung und episomale Säugervektoren). Andere Vektoren (z.B. nicht-episomale Säugervektoren) werden beim Einbringen in die Wirtszelle in das Genom einer Wirtszelle integriert und dadurch zusammen mit dem Wirtsgenom repliziert. Zudem können bestimmte Vektoren die Expression von Genen, mit denen sie funktionsfähig verbunden sind, steuern. Diese Vektoren werden hier als "Expressionsvektoren" bezeichnet. Gewöhnlich haben Expressionsvektoren, die für DNA-Rekombinationstechniken

geeignet sind, die Form von Plasmiden. In der vorliegenden Beschreibung können "Plasmid" und "Vektor" austauschbar verwendet werden, da das Plasmid die am häufigsten verwendete Vektorform ist. Die Erfindung soll jedoch diese anderen Expressionsvektorformen, wie virale Vektoren (z.B. replikationsdefiziente Retroviren, Adenoviren und adenoverwandte Viren), die ähnliche Funktionen ausüben, umfassen. Ferner soll der Begriff Vektor auch andere Vektoren, die dem Fachmann bekannt sind, wie Phagen, Viren, wie SV40, CMV, Baculovirus, Adenovirus, Transposons, IS-Elemente, Phasmide, Phagemide, Cosmide, lineare oder zirkuläre DNA, umfassen.

Die erfindungsgemäßen rekombinanten Expressionsvektoren umfassen eine erfindungsgemäße Nukleinsäure oder ein erfindungsgemäßes Genkonstrukt in einer Form, die sich zur Expression der Nukleinsäure in einer Wirtszelle eignet, was bedeutet, dass die rekombinanten Expressionsvektoren eine oder mehrere Regulationssequenzen, ausgewählt auf der Basis der zur Expression zu verwendenden Wirtszellen, die mit der zu exprimierenden Nukleinsäuresequenz funktionsfähig verbunden ist, umfasst. In einem rekombinanten Expressionsvektor bedeutet "funktionsfähig verbunden", dass die Nukleotidsequenz von Interesse derart an die Regulationssequenz(en) gebunden ist, dass die Expression der Nukleotidsequenz möglich ist und sie aneinander gebunden sind, so dass beide Sequenzen die vorhergesagte, der Sequenz zugeschriebene Funktion erfüllen (z.B. in einem In-vitro-Transkriptions-/Translationssystem oder in einer Wirtszelle, wenn der Vektor in die Wirtszelle eingebracht wird). Der Begriff "Regulationssequenz" soll Promotoren, Enhancer und andere Expressionskontrollelemente (z.B. Polyadenylierungssignale) umfassen. Diese Regulationssequenzen sind z.B. beschrieben in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), oder siehe: Gruber und Crosby, in: Methods in Plant Molecular Biology and Biotechnolgy, CRC Press, Boca Raton, Florida, Hrsgb.: Glick und Thompson, Kapitel 7, 89-108, einschließlich der Literaturstellen darin. Regulationssequenzen umfassen solche, welche die konstitutive Expression einer Nukleotidsequenz in vielen Wirtszelltypen steuern, und solche, welche die direkte Expression der Nukleotidsequenz nur in bestimmten Wirtszellen unter bestimmten Bedingungen steuern. Der Fachmann weiß, dass die Gestaltung des Expressionsvektors von Faktoren, wie der Auswahl der zu transformierenden Wirtszelle, dem Ausmaß der Expression des gewünschten Proteins usw., abhängen kann. Die erfindungsgemäßen Expressionsvektoren können in Wirtszellen eingebracht werden, um dadurch Proteine oder Peptide, einschließlich Fusionsproteinen oder -peptiden, herzustellen, die von den Nukleinsäuren, wie hier

beschrieben, kodiert werden (z.B. Desaturasen, mutante Formen von Desaturasen, Fusionsproteine usw.).

Die erfindungsgemäßen rekombinanten Expressionsvektoren können zur Expression von Desaturasen und Elongasen in prokaryotischen oder eukaryotischen Zellen gestaltet sein. Beispielsweise können Desaturasegene in bakteriellen Zellen, wie C. glutamicum, Insektenzellen (unter Verwendung von Baculovirus-Expressionsvektoren), Hefe- und anderen Pilzzellen (siehe Romanos, M.A., et al. (1992) "Foreign gene expression in yeast: a review", Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Hrsgb., S. 396-428: Academic Press: San Diego; und van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Hrsgb., S. 1-28, Cambridge University Press: Cambridge), Algen (Falciatore et al., 1999, Marine Biotechnology.1, 3:239-251), Ciliaten der Typen: Holotrichia, Peritrichia, Spirotrichia, Suctoria, Tetrahymena, Paramecium, Colpidium, Glaucoma, Platyophrya, Potomacus, Desaturaseudocohnilembus, Euplotes, Engelmaniella und Stylonychia, insbesondere der Gattung Stylonychia lemnae, mit Vektoren nach einem Transformationsverfahren, wie beschrieben in WO 98/01572, sowie Zellen vielzelliger Pflanzen (siehe Schmidt, R. und Willmitzer, L. (1988) "High efficiency *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" Plant Cell Rep.:583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, Kapitel 6/7, S.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press (1993), 128-43; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225 (und darin zitierte Literaturstellen)) oder Säugerzellen exprimiert werden. Geeignete Wirtszellen werden ferner erörtert in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Der rekombinante Expressionsvektor kann alternativ, zum Beispiel unter Verwendung von T7-Promotor-Regulationssequenzen und T7-Polymerase, in vitro transkribiert und translatiert werden.

Die Expression von Proteinen in Prokaryoten erfolgt meist mit Vektoren, die konstitutive oder induzierbare Promotoren enthalten, welche die Expression von Fusions- oder nicht-Fusionsproteinen steuern. Fusionsvektoren fügen eine Reihe von Aminosäuren an ein darin kodiertes Protein an, gewöhnlich am Aminoterminus des rekombinanten Proteins, aber auch am C-Terminus

oder fusioniert innerhalb geeigneter Bereiche in den Proteinen. Diese Fusionsvektoren haben gewöhnlich drei Aufgaben: 1) die Verstärkung der Expression von rekombinantem Protein; 2) die Erhöhung der Löslichkeit des rekombinanten Proteins und 3) die Unterstützung der Reinigung des rekombinanten Proteins durch Wirkung als Ligand bei der Affinitätsreinigung. Bei Fusions-Expressionsvektoren wird oft eine proteolytische Spaltstelle an der Verbindungsstelle der Fusionseinheit und des rekombinanten Proteins eingebracht, so dass die Abtrennung des rekombinanten Proteins von der Fusionseinheit nach der Reinigung des Fusionsproteins möglich ist. Diese Enzyme und ihre entsprechenden Erkennungssequenzen umfassen Faktor Xa, Thrombin und Enterokinase.

Typische Fusions-Expressionsvektoren sind u.a. pGEX (Pharmacia Biotech Inc; Smith, D.B., und Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) und pRIT5 (Pharmacia, Piscataway, NJ), bei denen Glutathion-S-Transferase (GST), Maltose E-bindendes Protein bzw. Protein A an das rekombinante Zielprotein fusioniert wird. Bei einer Ausführungsform ist die Desaturase-kodierende Sequenz in einen pGEX-Expressionsvektor kloniert, so dass ein Vektor erzeugt wird, der ein Fusionsprotein kodiert, das vom N-Terminus zum C-Terminus GST-Thrombin-Spaltstelle-X-Protein umfasst. Das Fusionsprotein kann durch Affinitätschromatographie unter Verwendung von Glutathion-Agarose-Harz gereinigt werden. Rekombinante Desaturase, die nicht an GST fusioniert ist, kann durch Spaltung des Fusionsproteins mit Thrombin gewonnen werden.

Beispiele für geeignete induzierbare nicht-Fusions-E. coli-Expressionsvektoren sind u.a. pTrc (Amann et al. (1988) Gene 69:301-315) und pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Kalifornien (1990) 60-89). Die Zielgenexpression vom pTrc-Vektor beruht auf der Transkription durch Wirts-RNA-Polymerase von einem Hybrid-trp-lac-Fusionspromotor. Die Zielgenexpression aus dem pET 11d-Vektor beruht auf der Transkription von einem T7-gn10-lac-Fusions-Promotor, die von einer coexprimierten viralen RNA-Polymerase (T7 gn1) vermittelt wird. Diese virale Polymerase wird von den Wirtsstämmen BL21 (DE3) oder HMS174 (DE3) von einem residenten λ-Prophagen bereitgestellt, der ein T7 gn1-Gen unter der Transkriptionskontrolle des lacUV 5-Promotors birgt.

Andere in prokaryotischen Organismen geeignete Vektoren sind dem Fachmann bekannt, diese Vektoren sind beispielsweise in E. coli pLG338, pACYC184, die pBR-Reihe, wie pBR322, die pUC-Reihe, wie pUC18 oder pUC19, die M113mp-Reihe, pKC30, pRep4, pHS1, pHS2,

pPLc236, pMBL24, pLG200, pUR290, pIN-III^{113}-B1, λgt11 or pBdCI, in Streptomyces pIJ101, pIJ364, pIJ702 oder pIJ361, in Bacillus pUB110, pC194 oder pBD214, in Corynebacterium pSA77 oder pAJ667. Eine Strategie zur Maximierung der Expression von rekombinantem Protein ist die Expression des Proteins in einem Wirtsbakterium, dessen Fähigkeit zur proteolytischen Spaltung des rekombinanten Proteins gestört ist (Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Kalifornien (1990) 119-128). Eine weitere Strategie ist die Veränderung der Nukleinsäuresequenz der in einen Expressionsvektor zu inserierenden Nukleinsäure, so dass die einzelnen Codons für jede Aminosäure diejenigen sind, die vorzugsweise in einem zur Expression ausgewählten Bakterium, wie *C. glutamicum*, verwendet werden (Wada et al. (1992) Nucleic Acids Res. 20:2111-2118). Diese Veränderung der erfindungsgemäßen Nukleinsäuresequenzen erfolgt durch Standard-DNA-Synthesetechniken.

Bei einer weiteren Ausführungsform ist der Desaturase-Expressionsvektor ein Hefe-Expressionsvektor. Beispiele für Vektoren zur Expression in der Hefe S. cerevisiae umfassen pYeDesaturasec1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan und Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) sowie pYES2 (Invitrogen Corporation, San Diego, CA). Vektoren und Verfahren zur Konstruktion von Vektoren, die sich zur Verwendung in anderen Pilzen, wie den filamentösen Pilzen, eignen, umfassen diejenigen, die eingehend beschrieben sind in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., Hrsgb., S. 1-28, Cambridge University Press: Cambridge, oder in: More Gene Manipulations in Fungi [J.W. Bennet & L.L. Lasure, Hrsgb., S. 396-428: Academic Press: San Diego]. Weitere geeignete Hefevektoren sind beispielsweise pAG-1, YEp6, YEp13 oder pEMBLYe23.

Alternativ können die erfindungsgemäßen Desaturasen in Insektenzellen unter Verwendung von Baculovirus-Expressionsvektoren exprimiert werden. Baculovirus-Vektoren, die zur Expression von Proteinen in gezüchteten Insektenzellen (z.B. Sf9-Zellen) verfügbar sind, umfassen die pAc-Reihe (Smith et al. (1983) Mol. Cell Biol.. 3:2156-2165) und die pVL-Reihe (Lucklow und Summers (1989) Virology 170:31-39).

Die oben genannten Vektoren bieten nur einen kleinen Überblick über mögliche geeignete Vektoren. Weitere Plasmide sind dem Fachmann bekannt und sind zum Beispiel beschrieben in: Cloning

Vectors (Hrsgb. Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018).

Bei noch einer weiteren Ausführungsform wird eine erfindungsgemäße Nukleinsäure in Säugerzellen unter Verwendung eines Säuger-Expressionsvektors exprimiert. Unter Säugern werden im Sinne der Erfindung alle nicht-humanen Säuger verstanden. Beispiele für Säuger-Expressionsvektoren umfassen pCDM8 (Seed, B. (1987) Nature 329:840) und pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-195). Bei der Verwendung in Säugerzellen werden die Kontrollfunktionen des Expressionsvektors oft von viralen Regulationselementen bereitgestellt. Üblicherweise verwendete Promotoren stammen z.B. aus Polyoma, Adenovirus2, Cytomegalievirus und Simian Virus 40. Weitere geeignete Expressionssysteme für prokaryotische und eukaryotische Zellen siehe in den Kapiteln 16 und 17 von Sambrook, J., Fritsch, E.F., und Maniatis, T., Molecular Cloning: A Laboratory Manual, 2. Auflage, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Bei einer anderen Ausführungsform kann der rekombinante Säuger-Expressionsvektor die Expression der Nukleinsäure vorzugsweise in einem bestimmten Zelltyp steuern (z.B. werden gewebespezifische Regulationselemente zur Expression der Nukleinsäure verwendet). Gewebespezifische Regulationselemente sind im Fachgebiet bekannt. Nicht beschränkende Beispiele für geeignete gewebespezifische Promotoren sind u.a. der Albuminpromotor (leberspezifisch; Pinkert et al. (1987) Genes Dev. 1:268-277), lymphoidspezifische Promotoren (Calame und Eaton (1988) Adv. Immunol. 43:235-275), insbesondere Promotoren von T-Zellrezeptoren (Winoto und Baltimore (1989) EMBO J. 8:729-733) und Immunglobulinen (Banerji et al. (1983) Cell 33:729-740; Queen und Baltimore (1983) Cell 33:741-748), neuronspezifische Promotoren (z.B. Neurofilament-Promotor; Byrne und Ruddle (1989) PNAS 86:5473-5477), pankreasspezifische Promotoren (Edlund et al., (1985) Science 230:912-916) und milchdrüsenspezifische Promotoren (z.B. Milchserum-Promotor; US-Patent Nr. 4,873,316 und Europäische Patentanmeldung-Veröffentlichung Nr. 264,166). Auch entwicklungsregulierte Promotoren sind umfasst, z.B. die hox-Promotoren der Maus (Kessel und Gruss (1990) Science 249:374-379) und der Fetoprotein-Promotor (Campes und Tilghman (1989) Genes Dev. 3:537-546).

Bei einer weiteren Ausführungsform können die erfindungsgemäßen Desaturasen in einzelligen Pflanzenzellen (wie Algen), siehe Falciatore et al., 1999, Marine Biotechnology 1 (3):239-251 und darin zitierte Literaturangaben, und Pflanzenzellen aus

höheren Pflanzen (z.B. Spermatophyten, wie Feldfrüchten) exprimiert werden. Beispiele für Pflanzen-Expressionsvektoren umfassen solche, die eingehend beschrieben sind in: Becker, D., Kemper, E., Schell, J., und Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1195-1197; und Bevan, M.W. (1984) "Binary Agrobacterium vectors for plant transformation", Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Bd. 1, Engineering and Utilization, Hrsgb.: Kung und R. Wu, Academic Press, 1993, S. 15-38.

Eine Pflanzen-Expressionskassette enthält vorzugsweise Regulationssequenzen, welche die Genexpression in Pflanzenzellen steuern können und funktionsfähig verbunden sind, so dass jede Sequenz ihre Funktion, wie Termination der Transkription, erfüllen kann, beispielsweise Polyadenylierungssignale. Bevorzugte Polyadenylierungssignale sind diejenigen, die aus Agrobacterium tumefaciens-t-DNA stammen, wie das als Octopinsynthase bekannte Gen 3 des Ti-Plasmids pTiACH5 (Gielen et al., EMBO J. 3 (1984) 835ff.) oder funktionelle Äquivalente davon, aber auch alle anderen in Pflanzen funktionell aktiven Terminatoren sind geeignet.

Da die Pflanzengenexpression sehr oft nicht auf Transkriptionsebenen beschränkt ist, enthält eine Pflanzen-Expressionskassette vorzugsweise andere funktionsfähig verbunden Sequenzen, wie Translationsenhancer, beispielsweise die Overdrive-Sequenz,, welche die 5'-untranslatierte Leader-Sequenz aus Tabakmosaikvirus, die das Protein/RNA-Verhältnis erhöht, enthält (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711).

Die Pflanzengenexpression muss funktionsfähig mit einem geeigneten Promotor verbunden sein, der die Genexpression auf rechtzeitige, zell- oder gewebespezifische Weise durchführt. Bevorzugt sind Promotoren, welche die konstitutive Expression herbeiführen (Benfey et al., EMBO J. 8 (1989) 2195-2202), wie diejenigen, die von Pflanzenviren stammen, wie 35S CAMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (siehe auch US 5352605 und WO 84/02913) oder Pflanzenpromotoren, wie der in US 4,962,028 beschriebene der kleinen Untereinheit der Rubisco.

Andere bevorzugte Sequenzen für die Verwendung zur funktionsfähigen Verbindung in Pflanzengenexpressions-Kassetten sind Targeting-Sequenzen, die zur Steuerung des Genproduktes in sein entsprechendes Zellkompartiment notwendig sind (siehe eine Übersicht in Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423

und darin zitierte Literaturstellen), beispielsweise in die Vakuole, den Zellkern, alle Arten von Plastiden, wie Amyloplasten, Chloroplasten, Chromoplasten, den extrazellulären Raum, die Mitochondrien, das Endoplasmatische Retikulum, Ölkörper, Peroxisomen und andere Kompartimente von Pflanzenzellen.

Die Pflanzengenexpression lässt sich auch über einen chemisch induzierbaren Promotor erleichtern (siehe eine Übersicht in Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemisch induzierbare Promotoren eignen sich besonders, wenn gewünscht wird, dass die Genexpression auf zeitspezifische Weise erfolgt. Beispiele für solche Promotoren sind ein Salicylsäure-induzierbarer Promotor (WO 95/19443), ein Tetracyclin-induzierbarer Promotor (Gatz et al. (1992) Plant J. 2, 397-404) und ein Ethanol-induzierbarer Promotor.

Auch Promotoren, die auf biotische oder abiotische Stressbedingungen reagieren, sind geeignete Promotoren, beispielsweise der pathogeninduzierte PRP1-Gen-Promotor (Ward et al., Plant. Mol. Biol. 22 (1993) 361-366), der hitzeinduzierbare hsp80-Promotor aus Tomate (US 5,187,267), der kälteinduzierbare Alpha-amylase-Promotor aus Kartoffel (WO 96/12814) oder der durch Wunden induzierbare pinII-Promotor (EP-A-0 375 091).

Es sind insbesondere solche Promotoren bevorzugt, welche die Genexpression in Geweben und Organen herbeiführen, in denen die Lipid- und Ölbiosynthese stattfindet, in Samenzellen, wie den Zellen des Endosperms und des sich entwickelnden Embryos. Geeignete Promotoren sind der Napingen-Promotor aus Raps (US 5,608,152), der USP-Promotor aus Vicia faba (Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67), der Oleosin-Promotor aus Arabidopsis (WO 98/45461), der Phaseolin-Promotor aus Phaseolus vulgaris (US 5,504,200), der Bce4-Promotor aus Brassica (WO 91/13980) oder der Legumin-B4-Promotor (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2):233-9) sowie Promotoren, welche die samenspezifische Expression in Monokotyledonen-Pflanzen, wie Mais, Gerste, Weizen, Roggen, Reis usw. herbeiführen. Geeignete beachtenswerte Promotoren sind der lpt2- oder lpt1-Gen-Promotor aus Gerste (WO 95/15389 und WO 95/23230) oder die in WO 99/16890 beschriebenen (Promotoren aus dem Gersten-Hordein-Gen, dem Reis-Glutelin-Gen, dem Reis-Oryzin-Gen, dem Reis-Prolamin-Gen, dem Weizen-Gliadin-Gen, Weizen-Glutelin-Gen, dem Mais-Zein-Gen, dem Hafer-Glutelin-Gen, dem Sorghum-Kasirin-Gen, dem Roggen-Secalin-Gen).

Insbesondere kann die multiparallele Expression von erfindungsgemäßen Desaturasen allein oder in Kombination mit anderen desaturasen oder Elongasen gewünscht sein. Die Einführung solcher Expressionskassetten kann über eine simultane Transformation mehrerer einzelner Expressionskonstrukte erfolgen oder durch Kombination mehrerer Expressionskassetten auf einem Konstrukt. Auch können mehrere Vektoren mit mit jeweils mehreren Exopressionskassetten transformiert und auf die Wirtszelle übertragen werden.

Ebenfalls besonders geeignet sind Promotoren, welche die plastidenspezifische Expression herbeiführen, da Plastiden das Kompartiment sind, in dem die Vorläufer sowie einige Endprodukte der Lipidbiosynthese synthetisiert werden. Geeignete Promotoren, wie der virale RNA-Polymerase-Promotor, sind beschrieben in WO 95/16783 und WO 97/06250, und der clpP-Promotor aus Arabidopsis, beschrieben in WO 99/46394.

Die Erfindung stellt zudem einen rekombinanten Expressionsvektor bereit, umfassend ein erfindungsgemäßes DNA Molekül, das in Antisense-Richtung in den Expressionsvektor kloniert ist. d.h. das DNA-Molekül ist derart mit einer regulatorischen Sequenz funktionsfähig verbunden, dass die Expression (durch Transkription des DNA-Moleküls) eines RNA-Moleküls, das zur Desaturase-mRNA "Antisense" ist, ermöglicht wird. Es können Regulationssequenzen ausgewählt werden, die funktionsfähig mit einer in Antisense-Richtung klonierten Nukleinsäure verbunden sind und die kontinuierliche Expression des Antisense-RNA-Moleküls in einer Vielzahl von Zelltypen steuern, zum Beispiel können virale Promotoren und/oder Enhancer oder Regulationssequenzen ausgewählt werden, welche die konstitutive, gewebespezifische oder zelltypspezifische Expression von Antisense-RNA steuern. Der Antisense-Expressionsvektor kann in Form eines rekombinanten Plasmids, Phagemids oder attenuierten Virus vorliegen, in dem Antisense-Nukleinsäuren unter der Kontrolle eines hochwirksamen regulatorischen Bereichs produziert werden, dessen Aktivität durch den Zelltyp bestimmt werden kann, in den der Vektor eingebracht worden ist. Eine Erläuterung der Regulation der Genexpression mittels Antisense-Genen siehe in Weintraub, H., et al., Antisense-RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics, Bd. 1(1) 1986.

Ein weiterer Aspekt der Erfindung betrifft Wirtszellen, in die ein erfindungsgemäßer rekombinanter Expressionsvektor eingebracht worden ist. Die Begriffe "Wirtszelle" und "rekombinante Wirtszelle" werden hier untereinander austauschbar verwendet. Selbstverständlich betreffen diese Begriffe nicht nur die bestimmte

Zielzelle, sondern auch die Nachkommen oder potentiellen Nachkommen dieser Zelle. Da in aufeinanderfolgenden Generationen aufgrund von Mutation oder Umwelteinflüssen bestimmte Modifikationen auftreten können, sind diese Nachkommen nicht unbedingt mit der Parentalzelle identisch, sind jedoch immer noch vom Umfang des Begriffs, wie hier verwendet, umfasst.

Unter Rekombinant oder Transgen beispielsweise rekombinanten Expressionsvektor oder rekombinanten Wirt oder Wirtszellen im Sinne der Erfindung ist zu verstehen, dass die erfindungsgemäßen Nukleinsäuren und/oder deren natürliche Regulationssequenzen an 5' und 3'-Position der Nukleinsäuren nicht in ihrer natürlichen Umgebung sind, das heißt entweder wurde die Lage der Sequenzen im Herkunfstorganismus verändert oder in diesem wurden die Nukleinsäuresequenzen und/oder die Regulationssequenzen mutiert oder die erfindungsgemäßen Nukleinsäuresequenzen wurden in einen anderen Organismus als den Herkunfstorganismus verbracht oder deren Regulationssequenzen. Auch Kombinationen dieser Veränderungen sind möglich. Unter natürlicher Umgebung ist die Lage einer Nukleinsäuresequenz in einem Organismus zu verstehen, wie er in der Natur vorkommt.

Eine Wirtszelle kann eine prokaryotische oder eukaryotische Zelle sein. Zum Beispiel kann eine Desaturase in Bakterienzellen, wie C. glutamicum, Insektenzellen, Pilzzellen oder Säugerzellen (wie Chinesischer Hamster-Ovarzellen (CHO) oder COS-Zellen), Algen, Ciliaten, Pflanzenzellen, Pilzen oder anderen Mikroorganismen, wie C. glutamicum, exprimiert werden. Andere geeignete Wirtszellen sind dem Fachmann geläufig.

Vektor-DNA lässt sich in prokaryotische oder eukaryotische Zellen über herkömmliche Transformations- oder Transfektionstechniken einbringen. Die Begriffe "Transformation" und "Transfektion", Konjugation und Transduktion, wie hier verwendet, sollen eine Vielzahl von im Stand der Technik bekannten Verfahren zum Einbringen fremder Nukleinsäure (z.B. DNA) in eine Wirtszelle, einschließlich Calciumphosphat- oder Calciumchlorid-Copräzipitation, DEAE-Dextran-vermittelte Transfektion, Lipofektion, natürliche Kompetenz, chemisch vermittelter Transfer, Elektroporation oder Teilchenbeschuss, umfassen. Geeignete Verfahren zur Transformation oder Transfektion von Wirtszellen, einschließlich Pflanzenzellen, lassen sich finden in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2. Aufl., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) und anderen Labor-Handbüchern, wie Methods in Molecular Biology, 1995, Bd. 44, Agrobacterium

protocols, Hrsgb: Gartland und Davey, Humana Press, Totowa, New Jersey.

Über die stabile Transfektion von Säugerzellen ist bekannt, dass je nach verwendetem Expressionsvektor und verwendeter Transfektionstechnik nur ein kleiner Teil der Zellen die fremde DNA in ihr Genom integriert. Zur Identifikation und Selektion dieser Integranten wird gewöhnlich ein Gen, das einen selektierbaren Marker (z.B. Resistenz gegen Antibiotika) kodiert, zusammen mit dem Gen von Interesse in die Wirtszellen eingebracht. Bevorzugte selektierbare Marker umfassen solche, welche Resistenz gegen Medikamente, wie G418, Hygromycin und Methotrexat, verleihen, oder in Pflanzen solche, welche Resistenz gegen ein Herbizid, wie Glyphosphat oder Glufosinat, verleihen. Weitere geeignete Marker sind beispielsweise Marker, welche Gene kodieren, die an Biosynthesewegen von zum Beispiel Zuckern oder Aminosäuren beteiligt sind, wie ß-Galactodsidase, ura3 oder ilv2. Marker, welche Gene, wie Luziferase, gfp oder andere Fluoreszenzgene kodieren, sind ebenfalls geeignet. Diese Marker lassen sich in Mutanten verwenden, in denen diese Gene nicht funktionell sind, da sie beispielsweise mittels herkömmlicher Verfahren deletiert worden sind. Ferner können Marker, welche eine Nukleinsäure kodieren, die einen selektierbaren Marker kodiert, in eine Wirtszelle auf dem gleichen Vektor eingebracht werden, wie derjenige, der eine Desaturase kodiert, oder können auf einem gesonderten Vektor eingebracht werden. Zellen, die mit der eingebrachten Nukleinsäure stabil transfiziert worden sind, können zum Beispiel durch Medikamentenselektion identifiziert werden (z.B. überleben Zellen, die den selektierbaren Marker integriert haben, wohingegen die anderen Zellen absterben).

Zur Erzeugung eines homolog rekombinierten Mikroorganismus wird ein Vektor hergestellt, der zumindest einen Abschnitt eines Desaturasegens enthält, in den eine Deletion, Addition oder Substitution eingebracht worden ist, um dadurch das Desaturasegen zu verändern, z.B. funktionell zu disrumpieren. Dieses Desaturasegen ist vorzugsweise ein Phaeodactylum tricornutum Desaturasegen, es kann jedoch ein Homologon oder Analogon aus anderen Organismen, sogar aus einer Säuger-, Pilz- oder Insektenquelle verwendet werden. Bei einer bevorzugten Ausführungsform ist der Vektor so gestaltet, dass das endogene Desaturasegen bei homologer Rekombination funktionell disrumptiert wird (d.h. nicht länger ein funktionelles Protein kodiert, auch als Knock-out-Vektor bezeichnet). Alternativ kann der Vektor so gestaltet sein, dass das endogene Desaturasegen bei homologer Rekombination mutiert oder anderweitig verändert wird, aber immer noch ein funktionelles Protein kodiert (z.B. kann der stromaufwärts

gelegene regulatorische Bereich so verändert sein, dass dadurch die Expression der endogenen Desaturase verändert wird). Zur Erzeugung einer Punktmutation über homologe Rekombination können auch als Chimeraplasty bekannte DNA-RNA-Hybride verwendet werden, die aus Cole-Strauss et al., 1999, Nucleic Acids Research 27(5):1323-1330 und Kmiec, Gene therapy, 19999, American Scientist, 87(3):240-247 bekannt sind.

Im Vektor für die homologe Rekombination ist der veränderte Abschnitt des Desaturasegens an seinem 5'- und 3'-Ende von zusätzlicher Nukleinsäure des Desaturasegens flankiert, so dass homologe Rekombination zwischen dem exogenen Desaturasegen, das auf dem Vektor vorliegt, und einem endogenen Desaturasegen in einem Mikroorganismus oder einer Pflanze möglich ist. Die zusätzliche flankierende Desaturase-Nukleinsäure ist für eine erfolgreiche homologe Rekombination mit dem endogenen Gen hinreichend lang. Gewöhnlich sind im Vektor mehrere hundert Basenpaare bis zu Kilobasen flankierende DNA (sowohl am 5'- als auch am 3'-Ende) enthalten (eine Beschreibung von Vektoren zur homologen Rekombination siehe z.B. in Thomas, K.R., und Capecchi, M.R. (1987) Cell 51:503 oder der Rekombination in Physcomitrella patens auf cDNA-Basis in Strepp et al., 1998, Proc. Natl. Acad. Sci. USA 95 (8):4368-4373). Der Vektor wird in einen Mikroorganismus oder eine Pflanzenzelle (z.B. mittels Polyethylenglycol-vermittelter DNA) eingebracht, und Zellen, in denen das eingebrachte Desaturasegen mit dem endogenen Desaturasegen homolog rekombiniert ist, werden unter Verwendung im Fachgebiet bekannter Techniken selektiert.

Bei einer anderen Ausführungsform können rekombinante Organismen, wie Mikroorganismen, hergestellt werden, die ausgewählte Systeme enthalten, welche eine regulierte Expression des eingebrachten Gens ermöglichen. Der Einschluß eines Desaturasegens in einem Vektor, wobei es unter die Kontrolle des lac-Operons gebracht wird, ermöglicht z.B. die Expression des Desaturasegens nur in Gegenwart von IPTG. Diese Regulationssysteme sind im Fachgebiet bekannt.

Eine erfindungsgemäße Wirtszelle, wie eine prokaryotische oder eukaryotische Wirtszelle, in Kultur oder auf einem Feld wachsend, kann zur Produktion (d.h. Expression) einer Desaturase verwendet werden. In Pflanzen kann zusätzlich ein alternatives Verfahren durch direkten Transfer von DNA in sich entwickelnde Blüten über Elektroporation oder Gentransfer mittels Agrobacterium angewendet werden. Die Erfindung stellt folglich ferner Verfahren zur Produktion von Desaturasen unter Verwendung der erfindungsgemäßen Wirtszellen bereit. Bei einer Ausführungsform umfasst das

Verfahren die Anzucht der erfindungsgemäßen Wirtszelle (in die ein rekombinanter Expressionsvektor, der eine Desaturase kodiert, eingebracht worden ist, oder in deren Genom ein Gen eingebracht worden ist, das eine Wildtyp- oder veränderte Desaturase kodiert) in einem geeigneten Medium, bis die Desaturase produziert worden ist. Das Verfahren umfasst bei einer weiteren Ausführungsform das Isolieren der Desaturasen aus dem Medium oder der Wirtszelle.

Wirtszellen, die im Prinzip zum Aufnehmen der erfindungsgemäßen Nukleinsäure, des erfindungsgemäßen Genproduktes oder des erfindungsgemäßen Vektors geeignet sind, sind alle prokaryotischen oder eukaryotischen Organismen. Die vorteilhafterweise verwendeten Wirtsorganismen sind Organismen, wie Bakterien, Pilze, Hefen, Tier- oder Pflanzenzellen. Weitere vorteilhafte Organismen sind Tiere oder vorzugsweise Pflanzen oder Teile davon. Pilze, Hefen oder Pflanzen werden vorzugsweise verwendet, besonders bevorzugt Pilze oder Pflanzen, ganz besonders bevorzugt Pflanzen, wie Ölfruchtpflanzen, die große Mengen an Lipidverbindungen enthalten, wie Raps, Nachtkerze, Canola, Erdnuss, Lein, Soja, Safflor, Sonnenblume, Borretsch, oder Pflanzen, wie Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Baumwolle, Maniok, Pfeffer, Tagetes, Solanaceen-Pflanzen, wie Kartoffel, Tabak, Aubergine und Tomate, Vicia-Arten, Erbse, Alfalfa, Buschpflanzen (Kaffee, Kakao, Tee), Salix-Arten, Bäume (Ölplame, Kokosnuß) sowie ausdauernde Gräser und Futterfeldfrüchte. Besonders bevorzugte erfindungsgemäße Pflanzen sind Ölfruchtpflanzen, wie Soja, Erdnuss, Raps, Canola, Lein, Nachtkerze, Sonnenblume, Safflor, Bäume (Ölpalme, Kokosnuß).

D. Isolierte Desaturase

Ein weiterer Aspekt der Erfindung betrifft isolierte Desaturasen und biologisch aktive Teile davon. Ein "isoliertes" oder "gereinigtes" Protein oder ein biologisch aktiver Teil davon ist im wesentlichen frei von zellulärem Material, wenn es durch DNA-Rekombinationstechniken produziert wird, oder von chemischen Vorstufen oder andern Chemikalien, wenn es chemisch synthetisiert wird. Der Begriff "im wesentlichen frei von zellulärem Material" umfasst Desaturase-Präparationen, in denen das Protein von zellulären Komponenten der Zellen, in denen es natürlich oder rekombinant produziert wird, getrennt ist. Bei einer Ausführungsform umfasst der Ausdruck "im wesentlichen frei von zellulärem Material" Desaturase-Präparationen mit weniger als etwa 30 % (bezogen auf das Trockengewicht) nicht-Desaturase (hier auch als "verunreinigendes Protein" bezeichnet), stärker bevorzugt weniger als etwa 20 % nicht-Desaturase, noch stärker bevorzugt weniger als etwa 10 % nicht-Desaturase und am stärksten bevorzugt weniger als etwa

5 % nicht-Desaturase. Wenn die Desaturase oder ein biologisch aktiver Teil davon rekombinant hergestellt worden ist, ist sie/er auch im wesentlichen frei von Kulturmedium, d.h. das Kulturmedium macht weniger als etwa 20 %, stärker bevorzugt weniger als etwa 10 % und am stärksten bevorzugt weniger als etwa 5 % des Volumens der Proteinpräparation aus. Der Begriff "im wesentlichen frei von chemischen Vorstufen oder anderen Chemikalien" umfasst Desaturase-Präparationen, in denen das Protein von chemischen Vorstufen oder anderen Chemikalien getrennt ist, die an der Synthese des Proteins beteiligt sind. Bei einer Ausführungsform umfasst der Begriff "im wesentlichen frei von chemischen Vorstufen oder anderen Chemikalien" Desaturase-Präparationen mit weniger als etwa 30 % (bezogen auf das Trockengewicht) chemischen Vorstufen oder nicht-Desaturase-Chemikalien, stärker bevorzugt weniger als etwa 20 % chemischen Vorstufen oder nicht-Desaturase-Chemikalien, noch stärker bevorzugt weniger als etwa 10 % chemischen Vorstufen oder nicht-Desaturase-Chemikalien und am stärksten bevorzugt weniger als etwa 5 % chemischen Vorstufen oder nicht-Desaturase-Chemikalien. Bei bevorzugten Ausführungsformen weisen isolierte Proteine oder biologisch aktive Teile davon keine verunreinigenden Proteine aus dem gleichen Organismus auf, aus dem die Desaturase stammt. Diese Proteine werden gewöhnlich durch rekombinante Expression zum Beispiel Phaeodactylum tricornutum-Desaturase in Pflanzen wie Physcomitrella patens bzw. o.g. oder Mikroorganismen, beispielsweise Bakterien, wie E. coli, Bacillus subtilis, C. glutamicum, Pilzen, wie Mortierella, Hefe, wie Saccharomyces, oder Ciliaten wie Colpidium oder Algen wie Phaeodactylum hergestellt.

Eine erfindungsgemäße isolierte Desaturase oder ein Teil davon kann auch am Stoffwechsel von zum Aufbau von Zellmembranen in Phaeodactylum tricornutum notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen. Bei bevorzugten Ausführungsformen umfasst das Protein oder der Teil davon eine Aminosäuresequenz, die ausreichend homolog zu einer Aminosäuresequenz der SEQ ID NO: 2, 4, 6 oder 12 ist, dass das Protein oder der Teil davon die Fähigkeit, am Stoffwechsel von zum Aufbau von Zellmembranen in Phaeodactylum tricornutum notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilzunehmen, beibehält. Der Teil des Proteins ist vorzugsweise ein biologisch aktiver Teil, wie hier beschrieben. Bei einer weiteren bevorzugten Ausführungsform hat eine erfindungsgemäße Desaturase eine der in SEQ ID NO: 2, 4, 6 oder 12 gezeigten Aminosäuresequenzen. Bei einer weiteren bevorzugten Ausführungsform hat die Desaturase eine Aminosäuresequenz, die von einer Nukleotidsequenz kodiert wird, die, zum Beispiel unter stringenten Bedingungen, an eine Nukleotidsequenz der

SEQ ID NO: 1, 3, 5 oder 11 hybridisiert. Bei noch einer weiteren bevorzugten Ausführungsform hat die Desaturase eine Aminosäuresequenz, die von einer Nukleotidsequenz kodiert wird, die mindestens etwa 50 bis 60 %, vorzugsweise mindestens etwa 60 bis 70 %, stärker bevorzugt mindestens etwa 70 bis 80 %, 80 bis 90 %, 90 bis 95 % und noch stärker bevorzugt mindestens etwa 96 %, 97 %, 98 %, 99 % oder noch homologer zu einer der Aminosäuresequenzen der SEQ ID NO: 2, 4, 6 oder 18 ist. Die erfindungsgemäße bevorzugte Desaturase besitzt vorzugsweise auch mindestens eine der hier beschriebenen Desaturase-Aktivitäten. Zum Beispiel umfasst eine erfindungsgemäße bevorzugte Desaturase eine Aminosäuresequenz, die von einer Nukleotidsequenz kodiert wird, die, zum Beispiel unter stringenten Bedingungen, an eine Nukleotidsequenz der SEQ ID NO: 1, 3, 5 oder 11 hybridisiert und am Stoffwechsel von zum Aufbau von Zellmembranen in Phaeodactylum tricornutum notwendigen Verbindungen oder am Transport von Molekülen über diese Membranen teilnehmen kann oder eine Doppelbindung in eine Fettsäure mit ein, zwei, drei oder vier Doppelbindungen und einer Kettenlänge von C_{18}, C_{20} oder C_{22} einführt.

Bei anderen Ausführungsformen ist die Desaturase im wesentlichen homolog zu einer Aminosäuresequenz der SEQ ID NO: 2, 4 oder 6 und behält die funktionelle Aktivität des Proteins einer der Sequenzen der SEQ ID NO: 2, 4 oder 6 bei, ihre Aminosäuresequenz unterscheidet sich jedoch aufgrund von natürlicher Variation oder Mutagenese, wie eingehend im obigen Unterabschnitt I beschrieben. Bei einer weiteren Ausführungsform ist die Desaturase folglich ein Protein, das eine Aminosäuresequenz umfasst, die mindestens etwa 50 bis 60 %, vorzugsweise mindestens etwa 60 bis 70 % und stärker bevorzugt mindestens etwa 70 bis 80 %, 80 bis 90 %, 90 bis 95 % und am stärksten bevorzugt mindestens etwa 96 %, 97 %, 98 %, 99 % oder noch homologer zu einer vollständigen Aminosäuresequenz der SEQ ID NO: 2, 4 oder 6 ist und zumindest eine der hier beschriebenen Desaturase-Aktivitäten aufweist. Bei einer anderen Ausführungsform betrifft die Erfindung ein vollständiges Phaeodactylum tricornutum-Protein, das im wesentlichen homolog zu einer vollständigen Aminosäuresequenz der SEQ ID NO: 2, 4 oder 6 ist.

Biologisch aktive Teile einer Desaturase umfassen Peptide, umfassend Aminosäuresequenzen, die von der Aminosäuresequenz einer Desaturase hergeleitet sind, z.B. eine in SEQ ID NO: 2, 4 oder 6 gezeigte Aminosäuresequenz oder die Aminosäuresequenz eines Proteins, das zu einer Desaturase homolog ist, welche weniger Aminosäuren als die Vollängen-Desaturase oder das Vollängenprotein aufweisen, das zu einer Desaturase homolog ist, und zumindest eine Aktivität einer Desaturase aufweisen.

Gewöhnlich umfassen biologisch aktive Teile (Peptide, z.B. Peptide, die zum Beispiel 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 oder mehr Aminosäuren lang sind) eine Domäne oder ein Motiv mit mindestens einer Aktivität einer Desaturase. Überdies können andere biologisch aktive Teile, in denen andere Bereiche des Proteins deletiert sind, durch rekombinante Techniken hergestellt und bezüglich einer oder mehrerer der hier beschriebenen Aktivitäten untersucht werden. Die biologisch aktiven Teile einer Desaturase umfassen vorzugsweise ein/eine oder mehrere ausgewählte Domänen/Motive oder Teile davon mit biologischer Aktivität.

Desaturasen werden vorzugsweise durch DNA-Rekombinationstechniken hergestellt. Zum Beispiel wird ein das Protein kodierendes Nukleinsäuremolekül in einen Expressionsvektor (wie vorstehend beschrieben) kloniert, der Expressionsvektor wird in eine Wirtszelle (wie vorstehend beschrieben) eingebracht, und die Desaturase wird in der Wirtszelle exprimiert. Die Desaturase kann dann durch ein geeignetes Reinigungsschema mittels Standard-Proteinreinigungstechniken aus den Zellen isoliert werden. Alternativ zur rekombinanten Expression kann eine Desaturase, ein -Polypeptid, oder -Peptid mittels Standard-Peptidsynthesetechniken chemisch synthetisiert werden. Überdies kann native Desaturase aus Zellen (z.B. Endothelzellen) z.B. unter Verwendung eines Anti-Desaturase-Antikörpers isoliert werden, der durch Standardtechniken produziert werden kann, wobei eine erfindungsgemäße Desaturase oder ein Fragment davon verwendet wird.

Die Erfindung stellt auch chimäre Desaturase-Proteine oder Desaturase-Fusionsproteine bereit. Wie hier verwendet, umfasst ein "chimäres Desaturase-Protein" oder "Desaturase-Fusionsprotein" ein Desaturase-Polypeptid, das funktionsfähig an ein nicht-Desaturase-Polypeptid gebunden ist. Ein "Desaturase-Polypeptid" betrifft ein Polypeptid mit einer Aminosäuresequenz, die einer Desaturase entspricht, wohingegen ein "nicht-Desaturase-Polypeptid" ein Polypeptid mit einer Aminosäuresequenz betrifft, die einem Protein entspricht, das im wesentlichen nicht homolog zu der Desaturase ist, z.B. ein Protein, das sich vom der Desaturase unterscheidet und aus dem gleichen oder einem anderen Organismus stammt. Innerhalb des Fusionsproteins soll der Begriff "funktionsfähig verbunden" bedeuten, dass das Desaturase-Polypeptid und das nicht-Desaturase-Polypeptid so miteinander fusioniert sind, dass beide Sequenzen die vorhergesagte, der verwendeten Sequenz zugeschriebene Funktion erfüllen. Das nicht-Desaturase-Polypeptid kann an den N-Terminus oder den C-Terminus des Desaturase-Polypeptids fusioniert sein. Bei einer Ausführungsform ist das Fusionsprotein zum Beispiel

ein GST-Desaturase-Fusionsprotein, bei dem die Desaturase-Sequenzen an den C-Terminus der GST-Sequenzen fusioniert sind. Diese Fusionsproteine können die Reinigung der rekombinanten Desaturasen erleichtern. Bei einer weiteren Ausführungsform ist das Fusionsprotein eine Desaturase, die eine heterologe Signalsequenz an ihrem N-Terminus aufweist. In bestimmten Wirtszellen (z.B. Säuger-Wirtszellen) kann die Expression und/oder Sekretion einer Desaturase durch Verwendung einer heterologen Signalsequenz gesteigert werden.

Ein erfindungsgemäßes chimäres Desaturase-Protein oder Desaturase-Fusionsprotein wird durch Standard-DNA-Rekombinationstechniken hergestellt. Zum Beispiel werden DNA-Fragmente, die unterschiedliche Polypeptidsequenzen kodieren, gemäß herkömmlicher Techniken im Leseraster aneinander ligiert, indem beispielsweise glatte oder überhängende Enden zur Ligation, Restriktionsenzymspaltung zur Bereitstellung geeigneter Enden, Auffüllen kohäsiver Enden, wie erforderlich, Behandlung mit alkalischer Phosphatase, um ungewollte Verknüpfungen zu vermeiden, und enzymatische Ligation eingesetzt werden. Bei einer weiteren Ausführungsform kann das Fusionsgen durch herkömmliche Techniken, einschließlich DNA-Syntheseautomaten, synthetisiert werden. Alternativ kann eine PCR-Amplifizierung von Genfragmenten unter Verwendung von Ankerprimern durchgeführt werden, die komplementäre Überhänge zwischen aufeinanderfolgenden Genfragmenten erzeugen, die anschließend miteinander hybridisiert und reamplifiziert werden können, so dass eine chimäre Gensequenz erzeugt wird (siehe zum Beispiel Current Protocols in Molecular Biology, Hrsgb. Ausubel et al., John Wiley & Sons: 1992). Überdies sind viele Expressionsvektoren kommerziell erhältlich, die bereits eine Fusionseinheit (z.B. ein GST-Polypeptid) kodieren. Eine Desaturase-kodierende Nukleinsäure kann in einen solchen Expressionsvektor kloniert werden, so dass die Fusionseinheit im Leseraster mit dem Desaturase-Protein verbunden ist.

Homologe der Desaturase können durch Mutagenese, z.B. durch spezifische Punktmutation oder Verkürzung der Desaturase, erzeugt werden. Der Begriff "Homologe", wie hier verwendet, betrifft eine variante Form der Desaturase, die als Agonist oder Antagonist der Desaturase-Aktivität wirkt. Ein Agonist der Desaturase kann im wesentlichen die gleiche Aktivität wie die oder einen Teil der biologischen Aktivitäten der Desaturase beibehalten. Ein Antagonist der Desaturase kann eine oder mehrere Aktivitäten der natürlich vorkommenden Form der Desaturase durch zum Beispiel kompetitive Bindung an ein stromabwärts oder -aufwärts gelegenes Element der Stoffwechselkaskade für Zellmembrankomponenten, welche die Desaturase umfasst, oder durch Bindung an eine

Desaturase, welche den Transport von Verbindungen über Zellmembranen vermittelt, hemmen, wodurch die Translokation gehemmt wird.

Bei einer alternativen Ausführungsform können Homologe der Desaturase durch Sichten kombinatorischer Banken von Mutanten, z.B. Verkürzungsmutanten, der Desaturase hinsichtlich Desaturase-Agonisten- oder -Antagonisten-Aktivität identifiziert werden. Bei einer Ausführungsform wird eine variegierte Bank von Desaturase-Varianten durch kombinatorische Mutagenese auf Nukleinsäure-ebene erzeugt und durch eine variegierte Genbank kodiert. Eine variegierte Bank von Desaturase-Varianten kann z.B. durch enzymatische Ligation eines Gemisches von synthetischen Oligonukleotiden in Gensequenzen hergestellt werden, so dass sich ein degenerierter Satz potentieller Desaturase-Sequenzen als individuelle Polypeptide oder alternativ als Satz größerer Fusionsproteine (z.B. für das Phage-Display), die diesen Satz von Desaturase-Sequenzen enthalten, exprimieren lässt. Es gibt eine Vielzahl von Verfahren, die zur Herstellung von Banken potentieller Desaturase-Homologen aus einer degenerierten Oligonukleotidsequenz verwendet werden können. Die chemische Synthese einer degenerierten Gensequenz kann in einem DNA-Syntheseautomaten durchgeführt und das synthetische Gen dann in einen geeigneten Expressionsvektor ligiert werden. Die Verwendung eines degenerierten Satzes von Genen ermöglicht die Bereitstellung sämtlicher Sequenzen, die den gewünschten Satz an potentiellen Desaturase-Sequenzen kodieren, in einem Gemisch. Verfahren zur Synthese degenerierter Oligonukleotide sind im Fachgebiet bekannt (siehe z.B. Narang, S.A. (1983) Tetrahedron 39:3; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al., (1984) Science 198:1056; Ike et al. (1983) Nucleic Acids Res. 11:477).

Zusätzlich können Banken von Desaturase-Fragmenten zur Herstellung einer variegierten Population von Desaturase-Fragmenten für das Sichten und für die anschließende Selektion von Homologen einer Desaturase verwendet werden. Bei einer Ausführungsform kann eine Bank von Fragmenten der kodierenden Sequenz durch Behandeln eines doppelsträngigen PCR-Fragmentes einer kodierenden Desaturase-Sequenz mit einer Nuklease unter Bedingungen, unter denen Doppelstrangbrüche nur etwa einmal pro Molekül erfolgen, Denaturieren der doppelsträngigen DNA, Renaturieren der DNA unter Bildung doppelsträngiger DNA, welche Sense/Antisense-Paare von verschiedenen Produkten mit Doppelstrangbrüchen umfassen kann, Entfernen einzelsträngiger Abschnitte aus neu gebildeten Duplices durch Behandlung mit S1-Nuklease und Ligieren der resultierenden Fragmentbank in einen Expressionsvektor erzeugt werden. Mit diesem Verfahren kann eine Expressionsbank hergeleitet werden,

die N-terminale, C-terminale und interne Fragmente der Desaturase verschiedener Größen kodiert.

Im Fachgebiet sind mehrere Techniken für das Sichten von Genprodukten in kombinatorischen Banken, die durch Punktmutationen oder Verkürzung hergestellt worden sind, und für das Sichten von cDNA-Banken nach Genprodukten mit einer ausgewählten Eigenschaft bekannt. Diese Techniken lassen sich an das schnelle Sichtung der Genbanken anpassen, die durch kombinatorische Mutagenese von Desaturase-Homologen erzeugt worden sind. Die am häufigsten verwendeten Techniken zum Sichtung großer Genbanken, die einer Analyse mit hohem Durchsatz unterworfen werden können, umfassen gewöhnlich das Klonieren der Genbank in replizierbare Expressionsvektoren, Transformieren von geeigneten Zellen mit der resultierenden Vektorenbank und Exprimieren der kombinatorischen Gene unter Bedingungen, unter denen der Nachweis der gewünschten Aktivität die Isolation des Vektors, der das Gen kodiert, dessen Produkt nachgewiesen wurde, erleichtert. Recursive-Ensemble-Mutagenese (REM), eine neue Technik, die die Häufigkeit funktioneller Mutanten in den Banken erhöht, kann in Kombination mit den Sichtungstests zur Identifikation von Desaturase-Homologen verwendet werden (Arkin und Yourvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delgrave et al. (1993) Protein Engineering 6(3):327-331).

Eine weitere bekannte Technik zur Veränderung von katalytischen Eigenschaften von Enzymen bzw. deren codierenden Genen ist das "Gen-Shuffling" (siehe z.B. in Stemmer, PNAS 1994, 91: 10747-10751, WO9720078 oder WO9813487), das eine Kombination von Genfragmenten darstellt, wobei diese Neukombination zusätzlich noch durch fehlerhafte Polymerasekettenreaktionen variiert werden kann und somit eine hohe zu testende Sequenzdiversität schafft. Voraussetzung für den Einsatz eines solchen Ansatzes ist jedoch ein geeignetes Screeningsystem, um die erstellte Gendiversität auf Funktionalität zu überprüfen.

Insbesondere für die Sichtung von Desaturaseaktivitäten ist ein Sichtungsverfahren Voraussetzung, das PUFA-abhängig Enzymaktivität(en) erfaßt. Bzgl. Desaturaseaktivitäten mit Spezifität für PUFAs kann man in Mucor-Species, die durch bekannte Transformationsverfahren mit gewünschten Genkonstrukten transformierbar sind, die Toxizität von Arachidonsäure in Anwesenheit eines toxischen Metaboliten (hier: Salicylsäure oder Salicylsäurederivate) nutzen (Eroshin et al., Mikrobiologiya, Vol. 65, No.1 1996, Seiten 31-36), um eine wachstumsbasierte Erstsichtung durchzuführen. Resultierende Klone können dann einer Analyse ihrer Lipidinhaltstoffe mittels Gaschromatographie und

Massenspektroskopie unterzogen werden, um Edukte und Produkte in Art und Menge zu erfassen.

Bei einer weiteren Ausführungsform können Tests auf Zellbasis zur Analyse einer variegierten Desaturase-Bank unter Verwendung von weiteren im Fachgebiet bekannten Verfahren ausgenutzt werden.

E. Erfindungsgemäße Verwendungen und Verfahren

Die hier beschriebenen Nukleinsäuremoleküle, Proteine, Proteinhomologen, Fusionsproteine, Primer, Vektoren und Wirtszellen können bei einem oder mehreren der nachstehenden Verfahren verwendet werden: Identifikation von Phaeodactylum und verwandten Organismen, Kartierung der Genome von Organismen, die mit Phaeodactylum tricornutum verwandt sind, Identifikation und Lokalisierung von Phaeodactylum tricornutum-Sequenzen von Interesse, Evolutionsstudien, Bestimmung von Desaturase-Proteinbereichen, die für die Funktion notwendig sind, Modulation einer Desaturase-Aktivität; Modulation des Stoffwechsels einer oder mehrerer Zellmembrankomponenten; Modulation des Transmembrantransports einer oder mehrerer Verbindungen sowie Modulation der zellulären Produktion einer gewünschten Verbindung, wie einer Feinchemikalie. Die erfindungsgemäßen Desaturase-Nukleinsäuremoleküle haben eine Vielzahl von Verwendungen. Sie können zunächst zur Identifikation eines Organismus als Phaeodactylum tricornutum oder als naher Verwandter davon verwendet werden. Sie können auch zur Identifikation des Vorliegens von Phaeodactylum tricornutum oder eines Verwandten davon in einer Mischpopulation von Mikroorganismen verwendet werden. Die Erfindung stellt die Nukleinsäuresequenzen einer Reihe von Phaeodactylum tricornutum-Genen bereit; durch Sondieren der extrahierten genomischen DNA einer Kultur einer einheitlichen oder gemischten Population von Mikroorganismen unter stringenten Bedingungen mit einer Sonde, die einen Bereich eines Phaeodactylum tricornutum -Gens oder von Teilen davon überspannt, das für diesen Organismus einzigartig ist, kann man bestimmen, ob dieser Organismus vorliegt. Phaeodactylum tricornutum selbst werden zur kommerziellen Produktion mehrfach ungesättigter Säuren verwendet und eignen darüber hinaus zur PUFA-Produktion auch in anderen Organismen insbesondere wenn erreicht werden soll, dass resultierende PUFAs auch in die Triacylglycerolfraktion eingebaut werden sollen.

Ferner können die erfindungsgemäßen Nukleinsäure- und Proteinmoleküle als Marker für spezifische Bereiche des Genoms dienen. Dies ist nicht nur zur Kartierung des Genoms, sondern auch für funktionelle Phaeodactylum tricornutum-Proteinen geeignet. Zur Identifikation des Genombereichs, an den ein bestimmtes DNA-

bindendes Protein von Phaeodactylum tricornutum bindet, könnte das Phaeodactylum tricornutum-Genom zum Beispiel gespalten werden und die Fragmente mit dem DNA-bindenden Protein inkubiert werden. Diejenigen, die das Protein binden, können zusätzlich mit den erfindungsgemäßen Nukleinsäuremolekülen, vorzugsweise mit leicht nachweisbaren Markierungen, sondiert werden; die Bindung eines solchen Nukleinsäuremoleküls an das Genomfragment ermöglicht die Lokalisierung des Fragments auf der Genomkarte von Phaeodactylum tricornutum und erleichtert, wenn dies mehrmals mit unterschiedlichen Enzymen durchgeführt wird, eine rasche Bestimmung der Nukleinsäuresequenz, an die das Protein bindet. Die erfindungsgemäßen Nukleinsäuremoleküle können zudem ausreichend homolog zu den Sequenzen verwandter Arten sein, dass diese Nukleinsäuremoleküle als Marker für die Konstruktion einer genomischen Karte bei verwandten Pilzen oder Algen dienen können.

Die erfindungsgemäßen Desaturase-Nukleinsäuremoleküle eignen sich auch für Evolutions- und Proteinstruktur-Untersuchungen. Die Stoffwechsel- und Transportprozesse, an denen die erfindungsgemäßen Moleküle beteiligt sind, werden von vielen prokaryotischen und eukaryotischen Zellen genutzt; durch Vergleich der Sequenzen der erfindungsgemäßen Nukleinsäuremoleküle mit solchen, die ähnliche Enzyme aus anderen Organismen kodieren, kann der Evolutions-Verwandschaftsgrad der Organismen bestimmt werden. Entsprechend ermöglicht ein solcher Vergleich die Bestimmung, welche Sequenzbereiche konserviert sind und welche nicht, was bei der Bestimmung von Bereichen des Proteins hilfreich sein kann, die für die Enzymfunktion essentiell sind. Dieser Typ der Bestimmung ist für Proteinengineering-Untersuchungen wertvoll und kann einen Hinweis darauf geben, wieviel Mutagenese das Protein tolerieren kann, ohne die Funktion zu verlieren.

Die Manipulation der erfindungsgemäßen Desaturase-Nukleinsäuremoleküle kann zur Produktion von Desaturasen mit funktionellen Unterschieden zu den Wildtyp-Desaturasen führen. Die Effizienz oder Aktivität dieser Proteine kann verbessert sein, sie können in größeren Anzahlen als gewöhnlich in der Zelle zugegen sein, oder ihre Effizienz oder Aktivität kann verringert sein. Verbesserte Effizienz oder Aktivität bedeutet beispielsweise, dass das Enzym eine höhere Selektivität und/oder Aktivität, vorzugsweise eine mindestens 10 % höhere, besonders bevorzugt eine mindestens 20 % höhere Aktivität, ganz besonders bevorzugt eine mindestens 30 % höhere Aktivität als das ursprüngliche Enzym aufweist.

Es gibt eine Reihe von Mechanismen, durch die die Veränderung einer erfindungsgemäßen Desaturase die Ausbeute, Produktion und/oder Effizienz der Produktion einer Feinchemikalie, welche ein solches verändertes Protein enthält, direkt beeinflussen kann. Die Gewinnung von Feinchemikalien-Verbindungen aus Kulturen von Ciliaten, Algen oder Pilzen im großen Maßstab ist signifikant verbessert, wenn die Zelle die gewünschten Verbindungen sezerniert, da diese Verbindungen aus dem Kulturmedium (im Gegensatz zur Extraktion aus der Masse der gezüchteten Zellen) leicht gereinigt werden können. Ansonsten lässt sich die Reinigung verbessern, wenn die Zelle in vivo Verbindungen in einem spezialisierten Kompartiment mit einer Art Konzentrationsmechanismus speichert. Bei Pflanzen, die Desaturasen exprimieren, kann ein gesteigerter Transport zu besserer Verteilung innerhalb des Pflanzengewebes und der -organe führen. Durch Vergrößern der Anzahl oder der Aktivität von Transportermolekülen, welche Feinchemikalien aus der Zelle exportieren, kann es möglich sein, die Menge der produzierten Feinchemikalie, die im extrazellulären Medium zugegen ist, zu steigern, wodurch Ernte und Reinigung oder bei Pflanzen eine effizientere Verteilung erleichtert werden. Zur effizienten Überproduktion einer oder mehrerer Feinchemikalien sind dagegen erhöhte Mengen an Cofaktoren, Vorläufermolekülen und Zwischenverbindungen für die geeigneten Biosynthesewege erforderlich. Durch Vergrößern der Anzahl und/oder der Aktivität von Transporterproteinen, die am Import von Nährstoffen, wie Kohlenstoffquellen (d.h. Zuckern), Stickstoffquellen (d.h. Aminosäuren, Ammoniumsalzen), Phosphat und Schwefel, beteiligt sind, kann man die Produktion einer Feinchemikalie aufgrund der Beseitigung aller Einschränkungen des Nährstoffangebots beim Biosyntheseprozess verbessern. Fettsäuren, wie PUFAs, und Lipide, die PUFAs enthalten, sind selbst wünschenswerte Feinchemikalien; durch Optimieren der Aktivität oder Erhöhen der Anzahl einer oder mehrerer erfindungsgemäßer Desaturasen, die an der Biosynthese dieser Verbindungen beteiligt sind, oder durch Stören der Aktivität einer oder mehrerer Desaturasen, die am Abbau dieser Verbindungen beteiligt sind, kann man somit die Ausbeute, Produktion und/oder Effizienz der Produktion von Fettsäure- und Lipidmoleküle in Ciliaten, Algen, Pflanzen, Pilzen, Hefen oder anderen Mikroorganismen steigern.

Die Manipulation eines oder mehrerer erfindungsgemäßer Desaturase-Gene kann ebenfalls zu Desaturasen mit veränderten Aktivitäten führen, welche die Produktion einer oder mehrerer gewünschter Feinchemikalien aus Algen, Pflanzen, Ciliaten oder Pilzen indirekt beeinflussen. Die normalen biochemischen Stoffwechselprozesse führen z.B. zur Produktion einer Vielzahl an Abfallprodukten (z.B. Wasserstoffperoxid und andere reaktive

Sauerstoffspezies), die diese Stoffwechselprozesse aktiv stören können (z.B. nitriert Peroxynitrit bekanntlich Tyrosin-Seitenketten, wodurch einige Enzyme mit Tyrosin im aktiven Zentrum inaktiviert werden (Groves, J.T. (1999) Curr. Opin. Chem. Biol. 3(2);226-235)). Diese Abfallprodukte werden zwar üblicherweise ausgeschieden, aber die zur fermentativen Produktion im großen Maßstab verwendeten Zellen werden für die Überproduktion einer oder mehrerer Feinchemikalien optimiert und können somit mehr Abfallprodukte produzieren als für eine Wildtypzelle üblich. Durch Optimieren der Aktivität einer oder mehrerer erfindungsgemäßer Desaturasen, die am Export von Abfallmolekülen beteiligt sind, kann man die Lebensfähigkeit der Zelle verbessern und eine effiziente Stoffwechselaktivität aufrechterhalten. Auch das Vorliegen hoher intrazellulärer Mengen der gewünschten Feinchemikalie kann tatsächlich für die Zelle toxisch sein, so dass man durch Steigern der Fähigkeit der Zelle zur Sekretion dieser Verbindungen die Lebensfähigkeit der Zelle verbessern kann.

Die erfindungsgemäßen Desaturasen können ferner so manipuliert sein, dass die relativen Mengen verschiedener Lipid- und Fettsäuremoleküle verändert werden. Dies kann eine entscheidende Auswirkung auf die Lipidzusammensetzung der Zellmembran haben. Da jeder Lipidtyp unterschiedliche physikalische Eigenschaften hat, kann eine Veränderung der Lipidzusammensetzung einer Membran die Membranfluidität signifikant verändern. Änderungen der Membranfluidität können den Transport von Molekülen über die Membran beeinflussen, was, wie vorstehend erläutert, den Export von Abfallprodukten oder der produzierten Feinchemikalie oder den Import notwendiger Nährstoffe modifizieren kann. Diese Änderungen der Membranfluidität können auch die Integrität der Zelle entscheidend beeinflussen; Zellen mit vergleichsweise schwächeren Membranen sind anfälliger gegenüber abiotischen und biotischen Stressbedingungen, welche die Zelle beschädigen oder abtöten können. Durch Manipulieren von Desaturasen, die an der Produktion von Fettsäuren und Lipiden für den Membranaufbau beteiligt sind, so dass die resultierende Membran eine Membranzusammensetzung hat, die für die in den Kulturen, die zur Produktion von Feinchemikalien verwendet werden, herrschenden Umweltbedingungen empfänglicher sind, sollte ein größerer Anteil der Zellen überleben und sich vermehren. Größere Mengen an produzierenden Zellen sollten sich in größeren Ausbeuten, höherer Produktion oder Effizienz der Produktion der Feinchemikalie aus der Kultur manifestieren.

Die vorstehend genannten Mutagenesestrategien für Desaturasen, die zu erhöhten Ausbeuten einer Feinchemikalie führen sollen, sollen nicht beschränkend sein; Variationen dieser Strategien

sind dem Fachmann leicht ersichtlich. Unter Verwendung dieser Mechanismen und mithilfe der hier offenbarten Mechanismen können die erfindungsgemäßen Nukleinsäure- und Proteinmoleküle zur Erzeugung von Algen, Ciliaten, Pflanzen, Tieren, Pilzen oder anderen Mikroorganismen, wie C. glutamicum, verwendet werden, die mutierte Desaturase-Nukleinsäure- und Proteinmoleküle exprimieren, so dass die Ausbeute, Produktion und/oder Effizienz der Produktion einer gewünschten Verbindung verbessert wird. Diese gewünschte Verbindung kann ein beliebiges natürliches Produkt von Algen, Ciliaten, Pflanzen, Tieren, Pilzen oder Bakterien sein, welches die Endprodukte von Biosynthesewegen und Zwischenprodukte natürlich vorkommender Stoffwechselwege umfasst, sowie Moleküle, die im Stoffwechsel dieser Zellen nicht natürlich vorkommen, die jedoch von den erfindungsgemäßen Zellen produziert werden.

Eine weitere erfindungsgemäße Ausführungsform ist ein Verfahren zur Produktion von PUFAs, wobei das Verfahren das Züchten eines Organismus, der eine erfindungsgemäße Nukleinsäure, ein erfindungsgemäßes Genkonstrukt oder einen erfindungsgemäßen Vektor umfasst, welche ein Polypeptid kodieren, das C_{18}-, C_{20}- oder C_{22}-Fettsäuren mit mindestens zwei Doppelbindungen im Fettsäuremolekül um mindestens zwei Kohlenstoffatome unter Bedingungen, unter denen PUFAs in dem Organismus produziert werden, verlängert, umfasst. Durch dieses Verfahren hergestellte PUFAs lassen sich durch Ernten der Organismen entweder aus der Kultur, in der sie wachsen, oder von dem Feld, Aufbrechen und/oder Extrahieren des geernteten Materials mit einem organischen Lösungsmittel isolieren. Aus diesem Lösungsmittel kann das Öl, das Lipide, Phospholipide, Sphingolipide, Glycolipide, Triacylglycerine und/oder freie Fettsäuren mit höherem Gehalt an PUFAs enthält, isoliert werden. Durch basische oder saure Hydrolyse der Lipide, Phospholipide, Sphingolipide, Glycolipide, Triacylglycerine können die freien Fettsäuren mit höherem Gehalt an PUFAs isoliert werden. Ein höherer Gehalt an PUFAs bedeutet mindestens 5 %, vorzugsweise 10 %, besonders bevorzugt 20 %, ganz besonders bevorzugt 40 % mehr PUFAs als der ursprüngliche Organismus, der keine zusätzliche Nukleinsäure, die die erfindungsgemäße Desaturase kodiert, besitzt.

Vorzugsweise sind die durch dieses Verfahren produzierten PUFAs C_{18}- oder C_{20-22}-Fettsäuremoleküle mit mindestens zwei Doppelbindungen im Fettsäuremolekül, vorzugsweise drei, vier, bei Kombination mit einer weiteren Elongasen und einer Δ-4 Desaturase fünf oder sechs Doppelbindungen. Diese C_{18}- oder C_{20-22}-Fettsäuremoleküle lassen sich aus dem Organismus in Form eines Öls, Lipids oder einer freien Fettsäure isolieren. Geeignete Organismen sind

beispielsweise die vorstehend erwähnten. Bevorzugte Organismen sind transgene Pflanzen.

Eine erfindungsgemäße Ausführungsform sind Öle, Lipide oder Fettsäuren oder Fraktionen davon, die durch das oben beschriebene Verfahren hergestellt worden sind, besonders bevorzugt Öl, Lipid oder eine Fettsäurezusammensetzung, die PUFAs umfassen und von transgenen Pflanzen herrühren.

Eine weitere erfindungsgemäße Ausführungsform ist die Verwendung des Öls, Lipids oder der Fettsäurezusammensetzung in Futtermitteln, Nahrungsmitteln, Kosmetika oder Pharmazeutika.

Ein weiterer Erfindungsgegenstand ist ein Verfahren zur Identifikation eines Antagonisten oder Agonisten von Desaturasen, umfassend

a) in Kontaktbringen der Zellen, die das Polypeptid der vorliegenden Erfindung exprimieren, mit einem Kandidatenstoff;

b) Testen der Desaturaseaktivität;

c) Vergleichen der Desaturaseaktivität mit einer Standardaktivität in Abwesenheit des Kandidatenstoffs, wobei ein Anstieg der Desaturaseaktivität über den Standard anzeigt, daß der Kandidatenstoff ein Agonist und ein Verringerung der Desaturaseaktivität anzeigt, daß der Kandidatenstoff ein Antagonist ist.

Der genannte Kandidatenstoff kann ein chemisch synthetisierter oder mikrobiologisch produzierter Stoff sein und z.B. in Zellextrakten von z.B. Pflanzen, Tieren oder Mikroorganismen auftreten. Weiterhin kann der genannte Stoff zwar im Stand der Technik bekannt sein, aber bisher nicht bekannt sein als die Aktivität der Desaturasen steigernd oder repremierend. Das Reaktionsgemisch kann ein zellfreier Extrakt sein oder eine Zelle oder Zellkultur umfassen. Geeignete Methoden sind dem Fachmann bekannt und werden z.B. allgemein beschrieben in Alberts, Molecular Biology the cell, 3rd Edition (1994), z.B. Kapitel 17. Die genannten Stoffe können z.B. zu dem Reaktionsgemisch oder dem Kulturmedium zugegeben werden oder den Zellen injiziert werden oder auf eine Pflanze gesprüht werden.

Wenn eine Probe, die ein nach der erfindungsgemäßen Methode aktiven Stoff beinhaltet, identifiziert wurde, dann ist es entweder möglich, den Stoff direkt von der ursprünglichen Probe zu

isolieren oder man kann die Probe in verschiedene Gruppen teilen, z.B. wenn sie aus einer Vielzahl von verschiedenen Komponenten besteht, um so die Zahl der verschiedenen Substanzen pro Probe zu reduzieren und dann das erfindungsgemäße Verfahren mit einer solchen "Unterprobe" der ursprünglichen Probe zu wiederholen. Abhängig von der Komplexität der Probe können die oben beschriebenen Schritte mehrmals wiederholt werden, vorzugsweise bis die gemäß der erfindungsgemäßen Methode identifizierte Probe nur noch eine geringe Anzahl von Substanzen oder nur noch eine Substanz umfaßt. Vorzugsweise wird der gemäß der erfindungsgemäßen Methode identifizierte Stoff oder Derivate davon weiter formuliert, so, daß er für die Anwendung in der Pflanzenzüchtung oder Pflanzenzell- oder Gewebekultur geeignet ist.

Die Stoffe, die gemäß dem erfindungsgemäßen Verfahren getestet und identifiziert wurden, können sein: Expressionsbibliotheken, z.B. cDNA-Expressionsbibliotheken, Peptide, Proteine, Nukleinsäuren, Antikörper, kleine organische Stoffe, Hormone, PNAs oder ähnliches (Milner, Nature Medicin 1 (1995), 879-880; Hupp, Cell. 83 (1995), 237-245; Gibbs, Cell. 79 (1994), 193-198 und darin zitierte Referenzen). Diese Stoffe könne auch funktionelle Derivate oder Analogon der bekannten Inhibitoren oder Aktivatoren sein. Verfahren zur Herstellung von chemischen Derivaten oder Analogon sind dem Fachmann bekannt. Die genannten Derivate und Analogon können gemäß Verfahren nach dem Stand der Technik getestet werden. Weiterhin kann computergestütztes Design oder Peptidomimetics zur Herstellung geeigneter Derivate und Analogon verwendet werden. Die Zelle oder das Gewebe, die/das für das erfindungsgemäße Verfahren verwendet werden kann, ist vorzugsweise eine erfindungsgemäße Wirtszelle, Pflanzenzelle oder ein Pflanzengewebe, wie in den oben genannten Ausführungsformen beschrieben.

Entsprechend betrifft die vorliegende Erfindung auch einen Stoff, der gemäß den vorstehenden erfindungsgemäßen Verfahren identifiziert wurde. Der Stoff ist z.B. ein Homolog der erfindungsgemäßen Desaturasen. Homologe der Desaturasen können durch Mutagenese, z.B. durch Punktmutation oder Deletion der Desaturasen, erzeugt werden. Hierin verwendet wird der Begriff "Homolog" als eine variante Form der Desaturasen, die als Agonist oder Antagonist für die Aktivität der Desaturasen wirkt. Ein Agonist kann die im wesentlichen gleiche oder einen Teil der biologischen Aktivität der Desaturasen haben. Ein Antagonist der Desaturasen kann eine oder mehr Aktivitäten der natürlich vorkommenden Formen der Desaturasen inhibieren, z.B. kompetitiv an ein *Downstream* oder *Upstream* gelegenes Mitglied der Fettsäuresynthese-Stoffwechselwege, die die Desaturasen einschließen,

binden oder an Desaturasen binden und dabei die Aktivität reduzieren oder inhibieren.

Außerdem betrifft die vorliegende Erfindung auch ein Antikörper oder ein Fragment davon, wie sie hierin beschrieben werden, der die Aktivität der erfindungsgemäßen Desaturasen inhibiert.

Bei einem Aspekt betrifft die vorliegende Erfindung ein Antikörper, der spezifisch den erfindungsgemäßen oben beschriebenen Agonisten oder Antagonisten erkennt bzw. bindet.

Ein weiterer Aspekt betrifft eine Zusammensetzung, die den Antikörper, den nach dem erfindungsgemäßen Verfahren identifizierten Stopp oder das Antisense-Molekül umfaßt.

In einer weiteren Ausführungsform betrifft die vorliegende Erfindung ein Kit, umfassend die erfindungsgemäße Nukleinsäure, das erfindungsgemäße Genkonstrukt, die erfindungsgemäße Aminosäuresequenz, das erfindungsgemäße Antisense-Nukleinsäuremolekül, den erfindungsgemäßen Antikörper und/oder Zusammensetzung, einen Antagonisten oder Agonisten, der nach dem erfindungsgemäßen Verfahren hergestellt wurde, und/oder erfindungsgemäße Öle, Lipide und/oder Fettsäuren oder eine Fraktion davon. Ebenso kann das Kit die erfindungsgemäßen Wirtszellen, Organismen, Pflanzen oder Teile davon, erntbare Teile der erfindungsgemäßen Pflanzen oder Vermehrungsmaterial oder aber auch den erfindungsgemäßen Antagonisten oder Agonisten umfassen. Die Komponenten des Kits der vorliegenden Erfindung können in geeigneten Containern, beispielsweise mit oder in Puffern oder anderen Lösungen verpackt sein. Ein oder mehr der genannten Komponenten können in ein und demselben Container verpackt sein. Zusätzlich oder alternativ können ein oder mehr der genannten Komponenten z.B. auf einer festen Oberfläche adsorbiert sein, z.B. Nitrozellulosefilter, Glasplatten, Chips, Nylonmembranen oder Mikrotiterplatten. Das Kit kann für jede der hierin beschriebenen Methoden und Ausführungsformen verwendet werden, z.B. für die Produktion von Wirtszellen, transgenen Pflanzen, zur Detektion von homologen Sequenzen, zur Identifikation von Antagonisten oder Agonisten usw. Weiterhin kann das Kit Anleitungen für die Verwendung des Kits für eine der genannten Anwendungen enthalten.

Diese Erfindung wird durch die nachstehenden Beispiele weiter veranschaulicht, die nicht als beschränkend aufgefaßt werden sollten. Der Inhalt sämtlicher in dieser Patentanmeldung zitierten Literaturstellen, Patentanmeldungen, Patente und

veröffentlichten Patentanmeldungen ist hier durch Bezugnahme aufgenommen.

Beispielteil

Beispiel 1: Allgemeine Verfahren

a) Allgemeine Klonierungsverfahren:

Klonierungsverfahren, wie beispielsweise Restriktionsspaltungen, Agarosegelelektrophorese, Reinigung von DNA-Fragmenten, Transfer von Nukleinsäuren auf Nitrocellulose- und Nylonmembranen, Verbindung von DNA-Fragmenten, Transformation von Escherichia coli- und Hefe-Zellen, Anzucht von Bakterien und Sequenzanalyse rekombinanter DNA, wurden durchgeführt wie beschrieben in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) oder Kaiser, Michaelis und Mitchell (1994) "Methods in Yeast Genetics" (Cold Spring Harbor Laboratory Press: ISBN 0-87969-451-3). Die Transformation und Anzucht von Algen, wie Chlorella oder Phaeodactylum werden durchgeführt wie beschrieben von El-Sheekh (1999), Biologia Plantarum 42:209-216; Apt et al. (1996) Molecular and General Genetics 252 (5):872-9.

b) Chemikalien

Die verwendeten Chemikalien wurden, wenn im Text nicht anders angegeben, in p. A.-Qualität von den Firmen Fluka (Neu-Ulm), Merck (Darmstadt), Roth (Karlsruhe), Serva (Heidelberg) und Sigma (Deisenhofen) bezogen. Lösungen wurden unter Verwendung von reinem pyrogenfreiem Wasser, im nachstehenden Text als H_2O bezeichnet, aus einer Milli-Q-Wassersystem-Wasserreinigungsanlage (Millipore, Eschborn) hergestellt. Restriktionsendonukleasen, DNA-modifizierende Enzyme und molekularbiologische Kits wurden bezogen von den Firmen AGS (Heidelberg), Amersham (Braunschweig), Biometra (Göttingen), Boehringer (Mannheim), Genomed (Bad Oeynhausen), New England Biolabs (Schwalbach/Taunus), Novagen (Madison, Wisconsin, USA), Perkin-Elmer (Weiterstadt), Pharmacia (Freiburg), Qiagen (Hilden) und Stratagene (Amsterdam, Niederlande). Wenn nicht anders angegeben, wurden sie nach den Anweisungen des Herstellers verwendet.

c) Zellmaterial

Die erfindungsgemäßen isolierten Nukleinsäuresequenzen sind im Genom eines Phaeodactylum tricornutum UTEX646-Stammes enthalten, der über die Algensammlung der University of Texas, Austin verfügbar ist.

Phaeodactylum tricornutum wurde bei 25°C mit einem Licht/Dunkel Rhythmus von 14:10 Stunden bei 22°C und 35 microEinstein (entspricht micromol Photonen pro Quadratmeter und Sekunde) in Glasröhren kultiviert, die von unten mit Luft begast wurden.

Als Kulturmedium für Phaeodactylum tricornutum wurde das f/2 Kulturmedium mit 10 % organischen Medium nach Guillard, R.R.L. verwendet (1975; Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L. and Chanley, M.H. (Eds.) Culture of marine Invertebrate animals, NY Plenum Press, pp. 29-60.): Es enthält

995,5 ml Seewasser (artifiziell)
1 ml $NaNO_3$ (75 g/l), 1 ml NaH_2PO_4 (5 g/l), 1 ml Spurenelementelösung, 1 ml Tris/Cl pH 8.0, 0.5 ml f/2 Vitaminlösung

Spurenelementelösung: Na_2EDTA (4,36 g/l), $FeCl_3$ (3,15 g/l), Primäre Spurenelemente: CuSO4 (10 g/l), $ZnSO_4$ (22 g/l), $CoCl_2$ (10 g/l), $MnCl_2$ (18 g/l), NaMoO4 (6,3 g/l)
f/2 Vitaminlösung: Biotin: 10 mg/l, Thiamin 200 mg/l, Vit B12 0,1 mg/l
org-Medium: Na-Acetat (1 g/l), Glucose (6 g/l), Na-Succinat (3 g/l), Bacto-Trypton (4 g/l), Hefe-Extrakt (2 g/l)

Beispiel: 2 Isolierung von Gesamt-DNA aus Phaeodactylum tricornutum UTEX646 für Hybridisierungsexperimente

Die Einzelheiten der Isolierung von Gesamt-DNA betreffen die Aufarbeitung von Pflanzenmaterial mit einem Frischgewicht von einem Gramm.

CTAB-Puffer: 2 % (Gew./Vol.) N-Acetyl-N,N,N-trimethylammonium-bromid (CTAB); 100 mM Tris-HCl, pH 8,0; 1,4 M NaCl; 20 mM EDTA.

N-Laurylsarkosin-Puffer: 10 % (Gew./Vol.) N-Laurylsarkosin; 100 mM Tris-HCl, pH 8,0; 20 mM EDTA.

Phaeodactylum tricornutum-Zellmaterial wurde unter flüssigem Stickstoff in einem Mörser verrieben, so dass ein feines Pulver erhalten wurde, und in 2 ml-Eppendorfgefäße überführt. Das ge-

frorene Pflanzenmaterial wurde dann mit einer Schicht von 1 ml Zersetzungspuffer (1 ml CTAB-Puffer, 100 ml N-Laurylsarkosin-Puffer, 20 ml ß-Mercaptoethanol und 10 ml Proteinase K-Lösung, 10 mg/ml) überschichtet und eine Stunde unter kontinuierlichem Schütteln bei 60°C inkubiert. Das erhaltene Homogenat wurde in zwei Eppendorfgefäße (2 ml) aufgeteilt und zweimal durch Schütteln mit dem gleichen Volumen Chloroform/Isoamylalkohol (24:1) extrahiert. Zur Phasentrennung wurde eine Zentrifugation bei 8000 x g und RT (= Raumtemperatur = ~ 23°C) jeweils 15 min lang durchgeführt. Die DNA wurde dann 30 min unter Verwendung von eiskaltem Isopropanol bei -70°C gefällt. Die gefällte DNA wurde bei 10000 g 30 min bei 4°C sedimentiert und in 180 ml TE-Puffer (Sambrook et al., 1989, Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) resuspendiert. Zur weiteren Reinigung wurde die DNA mit NaCl (1,2 M Endkonzentration) behandelt und erneut 30 min unter Verwendung des zweifachen Volumens an absolutem Ethanol bei -70°C gefällt. Nach einem Waschschritt mit 70 % Ethanol wurde die DNA getrocknet und anschließend in 50 ml H_2O + RNAse (50 mg/ml Endkonzentration) aufgenommen. Die DNA wurde über Nacht bei 4°C gelöst und die RNAse-Spaltung wurde anschließend 1 Std. bei 37°C durchgeführt. Die Aufbewahrung der DNA erfolgte bei 4°C.

Beispiel 3: Isolierung von Gesamt-RNA und poly(A)$^+$-RNA aus Pflanzen und Phaeodactylum tricornutum

Die Isolierung von Gesamt-RNA aus Pflanzen wie Lein und Raps etc. erfolgt nach einer bei Logemann et al beschriebenen Methode (1987, Anal. Biochem. 163, 21) isoliert. Aus Moos kann die Gesamt-RNA Protonema-Gewebe nach dem GTC-Verfahren (Reski et al., 1994, Mol. Gen. Genet., 244:352-359) gewonnen werden.

RNA Isolierung aus Phaeodactylum tricornutum:

Tiefgefrorene Algenproben (- 70°C) wurden in einem eiskaltem Mörser unter Flüssigstickstoff zu feinem Pulver zerreiben. 2 Volumen Homogenisationsmedium (12,024 g Sorbitol, 40,0 ml 1M Tris-HCl, pH 9 (0,2 M); 12,0 ml 5 M NaCl (0,3 M), 8,0 ml 250 mM EDTA, 761,0 mg EGTA, 40,0 ml 10 % SDS wurden auf 200 ml mit H_2O aufgefüllt und der pH auf 8,5 eingestellt) und 4 Volumen Phenol mit 0,2 % Mercaptoethanol wurden bei 40 bis 50°C unter gutem Mischen zu gefrorenem Zellpulver gegeben. Danach wurden 2 Volumen Chloroform hinzufügen und für 15 min kräftig gerührt. Es wurde 10 min bei 10000 g zentrifugiert und die wässrige Phase mit Phenol/Chloroform (2 Vol) und abschließend mit Chloroform extrahiert.

Das erhaltene Volumen der wässrigen Phase wurde mit 1/20 Vol 4 M Na-Acetat (pH 6) und 1 Vol Isopropanol (eiskalt) versetzet und die Nukleinsäuren bei -20°C über Nacht (= ÜN) gefällt. Anschließend wurde 30 min bei 10000 g zentrifugiert und der Überstand abgesogen. Es folgte ein Waschschritt mit 70 % EtOH und erneute Zentrifugation. Das Sediment wurde in Tris-Borat-Puffer (80 mM Tris-Borat-Puffer, 10 mM EDTA, pH 7,0) aufgenommen. Dann wurde der Überstand mit 1/3 Vol 8 M LiCl versetzt, gemischt 30 min bei 4°C inkubiert. Nach erneutem zentrifugieren wurde das Sediment mit 70 % Ethanol gewaschen, zentrifugiert und das Sediment in RNAse-freiem Wasser gelöst.

Die Isolierung von poly(A)$^+$-RNA erfolgte unter Verwendung von Dyna Beads® (Dynal, Oslo, Finnland) nach den Anweisungen im Protokoll des Herstellers.

Nach der Bestimmung der RNA- oder poly(A)$^+$-RNA-Konzentration wurde die RNA durch Zugabe von 1/10 Volumina 3 M Natriumacetat, pH 4,6, und 2 Volumina Ethanol gefällt und bei -70°C aufbewahrt.

Für die Analyse wurden jeweils 20 µg RNA in einem Formaldehyd-haltigen 1,5%igen Agarosegel aufgetrennt und auf Nylon Membranen (Hybond, Amersham) überführt. Der Nachweis spezifischer Transkripte wurde wie bei Amasino beschrieben durchgeführt ((1986) Anal. Biochem. 152, 304)).

Beispiel 4: Konstruktion der cDNA-Bank

Zur Konstruktion der cDNA-Bank aus Phaeodactylum tricornutum wurde die Erststrangsynthese unter Verwendung von Reverser Transkriptase aus Maus-Leukämie-Virus (Roche, Mannheim, Deutschland) und Oligo-d(T)-Primern, die Zweitstrangsynthese durch Inkubation mit DNA-Polymerase I, Klenow-Enzym und RNAse H-Spaltung bei 12°C (2 Std.), 16°C (1 Std.) und 22°C (1 STd.) erzielt. Die Reaktion wurde durch Inkubation bei 65°C (10 min) gestoppt und anschließend auf Eis überführt. Doppelsträngige DNA-Moleküle wurde mit T4-DNA-Polymerase (Roche, Mannheim) bei 37°C (30 min) mit glatten Enden versehen. Die Nukleotide wurden durch Phenol/Chloroform-Extraktion und Sephadex-G50-Zentrifugiersäulen entfernt. EcoRI/XhoI-Adapter (Pharmacia, Freiburg, Deutschland) wurden mittels T4-DNA-Ligase (Roche, 12°C, über Nacht) an die cDNA-Enden ligiert, mit XhoI nachgeschnitten und durch Inkubation mit Polynukleotidkinase (Roche, 37°C, 30 min) phosphoryliert. Dieses Gemisch wurde der Trennung auf einem Low-Melting-Agarose-Gel unterworfen. DNA-Moleküle über 300 Basenpaaren wurden aus dem Gel eluiert, Phenol-extrahiert, auf Elutip-D-Säulen (Schleicher und Schüll, Dassel, Deutschland) konzentriert und an Vektorarme

ligiert und in lambda-ZAP-Express-Phagen unter Verwendung des Gigapack Gold-Kits (Stratagene, Amsterdam, Niederlande) verpackt, wobei Material des Herstellers verwendet und seine Anweisungen befolgt wurden.

Beispiel 5: DNA-Sequenzierung und Computeranalyse

cDNA-Banken, wie im Beispiel 4 beschrieben, wurden zur DNA-Sequenzierung nach Standardverfahren, insbesondere durch das Kettenterminationsverfahren unter Verwendung des ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction-Kit (Perkin-Elmer, Weiterstadt, Deutschland), verwendet. Die Sequenzierung zufälliger, vereinzelter Klone wurde anschließend an die präparative Plasmidgewinnung aus cDNA-Banken über in vivo-Massen-excision und Retransformation von DH10B auf Agarplatten durchgeführt (Einzelheiten zu Material und Protokoll von Stratagene, Amsterdam, Niederlande). Plasmid-DNA wurde aus über Nacht gezüchteten E. coli-Kulturen, die in Luria-Brühe mit Ampicillin (siehe Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6)) gezüchtet worden waren, an einem Qiagen-DNA-Präparations-Roboter (Qiagen, Hilden) nach den Protokollen des Herstellers präpariert. Sequenzierprimer mit den folgenden Nukleotidsequenzen wurden verwendet:

5'-CAGGAAACAGCTATGACC-3'
5'-CTAAAGGGAACAAAAGCTG-3'
5'-TGTAAAACGACGGCCAGT-3'

Die Sequenzen wurden unter Verwendung des Standard-Softwarepakets EST-MAX, das kommerziell von Bio-Max (München, Deutschland) geliefert wird, prozessiert und annotiert. Durch Nutzung von Vergleichsalgorithmen und unter Verwendung der in SEQ ID NO: 8 dargestellten Suchsequenz wurde mithilfe des BLAST-Programms nach homologen Genen gesucht (Altschul et al. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.). Zwei Sequenzen aus Phaeodactylum tricornutum mit Homologien zur Suchsequenz aus Physcomitrella patens wurden eingehender charakterisiert.

Beispiel 5a: Isolation von Desaturasen aus Phaeodactylum tricornutum über Polymerase Kettenreaktion mithilfe degenerierter Oligonukleotide:

Mithilfe von publizierten Desaturasen können Motive identifiziert werden, die für Δ-5 und Δ-6 Desaturasen typisch sind. Im folgenden sind Oligonukleotidsequenzen mit möglichen Variationen dargestellt. Unter der Oligonukleotidsequenz ist im Ein-Buchstabencode die Aminosäure dargestellt, von der die Basenkombination abgeleitet werden kann. Z.B. bedeutet A/G, daß an dieser Position bei der Synthese des Bausteins statistisch gleichverteilt entweder ein A oder ein G in das Oligonukleotid eingebaut wird, da das von der korrespondierenden Aminosäure abgeleitete Basentriplett entweder ein AAA oder ein AAG sein kann. Die DNA Sequenz kann auch ein Inosin (i) enthalten, wenn die Bestimmung einer Base an dieser Position aufgrund des genetischen Codes drei oder vier unterschiedliche Basen erlaubt. Folgende Sequenzen und Primer können verwendet werden:

5'-Vorwärts-Primer:

```
F1a:  TGG TGG AA A/G TGG   AAi      CA T/C  AA
F1b:  TGG TGG AA A/G TGG   ACi      CA T/C  AA
F1a:   W   W   K      W    N/T        H     K/N
F1b:   W   W   K      W     K         H     K/N

F2a:   Gi TGG AA A/G GAi   A/C Ai CA T/C  AA
F2b:   Gi TGG AA A/G TTG   A/C Ai CA T/C  AA
F2a:  G/W  W   K     E/D   K/Q/N   H      K/N
F2b:  G/W  W   K      W    K/Q/N   H      K/N
F3a:  T A/T i    TTG  AAi  A/C A A/G  C/A G/A i  CA
F3b:  T A/T i    TTG  AAi  A/C A A/G  CAi        CA
F3a:  W          W    K/N  H/N        R/Q        H
F3b:  Y          W    K/N  H/N        R/Q        H
F4a:       GTi TGG A A/T G/A   GA A/G     CA A/G CA
F4b:       GTi TGG A A/T G/A   A/T A T/C  CA A/G CA
F4a:       V    W    K/M       E           Q     H
F4b:       V    W    K/M       N/Y         Q     H
F5a1:      CA T/C  TA T/C TGG AA A/G AA T/C CA G  C
F5a1:      CA T/C  TA T/C TGG AA A/G AA T/C CA A  C
F5a1:       H       Y      W   K      N      Q    H/Q
F6a:  TTG  TTG  AAi  A/C A A/G  AA i       CA T/C  AA
F6a:  W    W    K/N  H/N        K/N        H      K/N
```

3'- Reverse Primer

R1b:	GG A/G AA iAG G/A TG G/A TG T/C TC
R1b:	GG A/G AA iAA G/A TG G/A TG T/C TC
R1a:	P F L H H E
R1b:	P F F H H E
R2a1:	AA iAG A/G TG A/G TG iA C/T iA/G T/C TG
R2a2:	AA T/C AA A/G TG A/G TG iA C/T iA/G T/C TG
R2a1:	F L H H V/I V/A Q
R3a1:	AT iTG iGG A/G AA iAA A/G TG A/G TG
R3a2:	AT A/G TT iGG A/G AA iAA A/G TG A/G TG
R3a3:	AT iTG iGG A/G AA iAG A/G TG A/G TG
R3a4:	AT A/G TT iGG A/G AA iAG A/G TG A/G TG
R3a1:	I/M H/Q P F F H H
R3a2:	I/M N P F L H H
R4a1:	CT iGG A/G AA iA A/G A/G TG A/G TG
R4a2:	GA iGG A/G AA iA A/G A/G TG A/G TG
R4a3:	GT iGG A/G AA iA A/G A/G TG A/G TG
R4a1:	= T/R/S P F F/L H H
R5a1:	AA iAA A/G TG A/G TG T/C TC T/A/G AT T/C TG
R5a2:	AA iAG A/G TG A/G TG T/C TC T/A/G AT T/C TG
R5a1:	F F H H E I Q
R5a2:	F L H H E I Q
R6a1:	T iGG iA A/G iAA A/G TG A/G TG iAC
R6a1:	T iGG iA A/G iAG A/G TG A/G TG iAC
R6a1:	T/N P L F/L H H V

Aufgrund verschiedener Variationsmöglichkeiten sind viele abgeleitete Oligonukleotide möglich, jedoch überraschenderweise gefunden wurde, dass dargestellte Oligonukleotide besonders zur Isolation von Desaturasen geeignet sein können.

Die Primer können in allen Kombinationen für Polymerase Kettenreaktionen eingesetzt werden. Mithilfe einzelner Kombinationen konnten Desaturase-Fragmente isoliert, wenn nachfolgende Bedingungen berücksichtigt wurden: Für PCR Reaktionen wurden jeweils 10 nMol Primer und 10 ng einer durch in vivo Excision gewonnenen Plasmidbank eingesetzt. Die Plasmidbank konnte nach Protokollen des Herstellers (Stratagene) aus der Phagenbank isoliert werden. Die PCR-Reaktion wurde in einem Thermocycler (Biometra) mit der Pfu-DNA-Polymerase (Stratagene) und dem folgenden Temperaturprogramm durchgeführt: 3 min bei 96°C, gefolgt von 35 Zyklen mit 30 s bei 96°C, 30 s bei 55°C und 1 min bei 72°C. Dabei wurde die Anlagerungstemperatur nach dem ersten Schritt von 55°C schrittweise um je 3°C erniedrigt und nach dem fünften Zyklus eine Anlagerungstemperatur von 40°C beibehalten. Letztlich wurde

ein Zyklus mit 10 min bei 72°C durchgeführt und der Ansatz durch Kühlen auf 4°C beendet.

Die Primerkombination F6a und R4a2 sind im Text unterstrichen gekennzeichnet und konnten erfolgreich zur Isolierung eines Desaturasefragmentes genutzt werden. Das resultierende Fragment konnte durch Sequenzierung verifiziert werden und zeigte Homologien zu einer Desaturase mit der Genbank Accession Nr. T36617 aus Streptomyces coelicolor. Die Homologie wurde mithilfe des BLASTP Programmes erhalten. Der Vergleich ist in Figur 4 dargestellt. Es ergaben sich Identitäten von 34 % und eine Homologie von 43 % zu Sequenz T36617. Das DNA-Fragment wurde gemäß Beispiel 7 in einem Hybridisierungsexperiment zur Isolierung eines Vollängengens nach Standardbedingungen erfindungsgemäß eingesetzt.

Die Codierregion einer so isolierten DNA-Sequenz wurde durch Übersetzung des genetischen Codes in eine Polypeptidsequenz erhalten. In SEQ ID NO: 3 ist eine 1434 Basenpaare lange Sequenz dargestellt, die durch beschriebenes Verfahren isoliert werden konnte. Die Sequenz besitzt ein Startcodon in Position 1 bis 3 und ein Stopcodon in Position 1432-1434 und konnte in ein 477 Aminosäuren langes Polypeptid übersetzt werden. Durch Vergleich mit einer in WO 98 46763 beschriebenen Gensequenz wurde gefunden, dass ein nicht identisches aber homologes Fragment aus Phaeodactylum tricornutum codierend für 87 Aminosäuren vorbeschrieben wurde. Jedoch offenbart WO 98/46763 weder eine vollständige, funktionell aktive Desaturase noch Positions- oder Substratspezifiät. Dies wird auch dadurch deutlich, dass sowohl Homologien zur Δ-5, als auch zur Δ-6-Desaturase aus Mortierella alpina berichtet werden, ohne eine genaue Funktion festzulegen. Die erfindungsgemäße Sequenz hingegen codiert für eine funktionell aktive Δ-6-Acyl Lipid Desaturase.

Beispiel 6: Identifizierung von DNA Sequenzen codierend für Desaturasen aus Phaeodactylum tricornutum

Die Volllängensequenz der Δ-6-Acyl Lipid Desaturase Pp_des6 AJ222980 (NCBI Genbank Accession Nr.) aus dem Moos Physcomitrella patens (siehe auch Tabelle 1) sowie die Δ-12-acyl Lipid Desaturase Sequenz (Tabelle 1 siehe Ma_des12) aus Mortierella alpina AF110509 (AF110509 NCBI Genbank Accession Nr.) wurden für Sequenzvergleiche mithilfe des TBLASTN Suchalgorhythmus eingesetzt.

Die EST-Sequenzen PT0010070010R, PT001072031R sowie PT001078032R wurden zunächst aufgrund schwacher Homologien mit den Suchsequenzen aus Physcomitrella und Mortierella unter weiteren Kandidatengenen als Zielgen in Betracht gezogen. In Figur 1 und in Figur 2 sowie Figur 2a ist das Ergebnis der zwei gefundenen est-Sequenzen dargestellt. Die gefundenen Sequenzen sind Teil der erfindungsgemäßen Nukleinsäuren aus SEQ ID NO: 1 (Genname: Pt_des5, eigene Datenbank Nr. der Erfinder PT001078032R), SEQ ID NO: 5. (Genname: Pt_des12, eigene Datenbank NR. der Erfinder PT0010070010R) und SEQ ID NO: 11 (Genname: Pt_des12.2, eigene Datenbank des Erfinders PT001072031R). Buchstaben zeigen identische Aminosäuren an, während das Pluszeichen eine chemisch ähnliche Aminosäure bedeutet. Die Identitäten bzw. Homologien aller erfindungsgemäß gefundener Sequenzen gehen aus Tabelle 2 zusammenfassend hervor.

Desaturasen können Cytochrom b5 Domänen aufweisen, die auch in anderen nicht Desaturasen codierenden Genen vorkommen. Cytochrom b5 Domänen zeigen mithin hohe Homologien an, obwohl es sich um verschiedene Genfunktionen handelt. Desaturasen können schwach konservierter Bereiche lediglich als putative Kandidatengene identifiziert werden und müssen auf die Enzymaktivität und Positionsspezifität der enzymatischen Funktion hin geprüft werden. Beispielsweise zeigen auch verschiedene Hydroxylasen, Acetylenasen und Epoxygenasen ähnlich wie Desaturasen Histidin-Box Motive, so dass eine konkrete Funktion experimentell nachgewiesen werden muß und zusätzlich die Verifizierung der Doppelbindung erst eine sichere Enzymaktivität und Positionsspezifität einer Desaturase ermöglicht. Überraschenderweise wurde gefunden, dass erfindungsgemäße Δ-6- und Δ-5- Desaturase besonders geeignete Substratspezifitäten aufweisen und besonders geeignet sind, um in Kombination mit einer Δ-6-Elongase aus Physcomitrella zur Produktion von polyungesättigten Fettsäuren wie Arachidonsäure, Eicosapentaensäure und Docosahexaensäure genutzt werden können.

Die Sequenzierung des vollständigen cDNA Fragmentes aus Klon PT001078032R ergab eine 1652 Basenpaare lange Sequenz. Die Sequenz codiert für ein Polypeptid von 469 Aminosäuren dargestellt in SEQ ID NO: 2. Diese wurde erhalten durch Übersetzung des genetischen Codes aus SEQ ID NO: 1 mit einem Startcodon in Basenpaarposition 115-117 und mit einem Stopcodon in Basenpaarposition 1522-1524. Der Klon beinhaltet ein vollständiges Desaturase-Polypeptid, wie aus dem Sequenzvergleich in Figur 3 zu ersehen ist. Striche bedeuten identische Aminosäuren während Doppelpunkte und Einzelpunkte chemisch austauschbare, d.h. chemisch äquivalente Aminosäuren darstellen. Der Vergleich wurde mit der BLOSUM62 Austauschmatrix für Amino-

säuren nach Henikoff & Henikoff durchgeführt:((1992) Amino acid substitution matrices from protein blocks. Proc. Natl. Acad.Sci. USA 89: 10915-10919). Verwendete Parameter: Gap Weight: 8; Average Match: 2.912, Length Weight: 2, Average Mismatch: -2.003.

In Figur 6 und Figur 7 ist der Vergleich der MA_des12 Peptidsequenz mit den gefundenen Sequenzen dargestellt.

Die Sequenzierung des vollständigen cDNA Fragmentes aus Klon PT0010070010R ergab eine in SEQ ID NO: 5 dargestellte 1651 Basenpaare lange Sequenz mit einem Startcodon in Position 67-69 und einem Stopcodon in Position 1552-1554. Die erfindungsgemäße Polypetidsequenz ist in SEQ ID NO: 6 dargestellt.

Die Sequenzierung des vollständigen identifizierten cDNA Fragmentes aus Klon PT0010072031R ergab eine in SEQ ID NO: 11 dargestellte 1526 Basenpaare lange Sequenz mit einem Startcodon in Position 92-94 und einem Stopcodon in Position 1400-1402. Die erfindungsgemäße Polypetidsequenz ist in SEQ ID NO: 12 dargestellt.

In Tabelle 2 sind die Identitäten und Homologien erfindungsgemäßer Desaturasen untereinander und mit der Desaturase aus Physcomitrella patens und Mortierella alpina dargestellt. Die Angaben wurden mithilfe des Programms Bestfit unter gegebenen Parametern wie unten definiert als Teilprogramm folgender Software erhalten: Wisconsin Package Version 10.0 (Genetics Computer Group (GCG), Madison, Wisc., USA). Henikoff, S. and Henikoff, J.G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919.

Weiterhin ist in Figur 5 der Vergleich der Δ-6-acyl Lipid Desaturase aus Physcomitrella patens mit der Polypeptidsequenz des Klons Pt_des6 dargestellt.

Tabelle 2:

Homologie / Identität in %	Suchsequenz Pp_des6	Suchsequenz Ma_des12
Pt_des5	34.92/26.37	n.d.
Pt_des6	50.69/41.06	n.d.
Pt_des12	n.d.	48.58/38.92
Pt_des12.2	n.d.	48.37/41.60

n.d. = nicht durchgeführt

Mithilfe des Algorhythmus TBLASTN 2.0.10: Altschul et al 1997, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402 wurden über einen lokalen Datenbankvergleich Sequenzen mit höchster Sequenzhomologie bzw. Identität identifiziert. Die Ergebnisse sind in folgender Tabelle 2A dargestellt.

Tabelle 2A: Homologe mit den höchsten Sequenzhomologien bzw Identitäten zu erfindungsgemäßen Polypeptidsequenzen aus SEQ ID NO. 2, 4, 6 oder 12

Homologie / Identität (%)	Suchsequenz PT001070010R	Suchsequenz PT001072031R	Suchsequenz PT001078032R	Suchsequenz Pt_des6
L26296: Fad2 A. thaliana	50 % / 37 %	n.d.	n.d.	n.d.
U86072 Petroselinum crispum Fad2	n.d.	51/40	n.d.	n.d.
AL358652 L. major putative desaturase	n.d.	n.d.	45/30	n.d.
AB020032 M. alpina delta 6 desaturase	n.d.	n.d.	n.d.	53/38

Beispiel 7: Identifikation von Genen mittels Hybridisierung

Gensequenzen lassen sich zur Identifikation homologer oder heterologer Gene aus cDNA- oder genomischen Banken verwenden.

Homologe Gene (d.h. Voll-Längen-cDNA-Klone, die homolog sind, oder Homologen) lassen sich über Nukleinsäurehybridisierung unter Verwendung von beispielsweise cDNA-Banken isolieren: Insbesondere zur Isolierung von funktionell aktiven Voll-Längengenen der in SEQ ID NO: 3 gezeigten kann die Methode genutzt werden. Je nach der Häufigkeit des Gens von Interesse werden 100000 bis zu 1000000 rekombinante Bakteriophagen plattiert und auf eine Nylonmembran überführt. Nach der Denaturierung mit Alkali wurde die DNA auf der Membran z.B. durch UV-Vernetzung immobilisiert. Die Hybridisierung erfolgt bei hoch-stringenten Bedingungen. In wässriger Lösung werden die Hybridisierung und die Waschschritte bei einer Ionenstärke von 1 M NaCl und einer Temperatur von 68°C durchgeführt. Hybridisierungssonden wurden z.B. durch Markierung mittels radioaktiver (^{32}P-) Nicktranskription (High Prime, Roche,

Mannheim, Deutschland) hergestellt. Die Signale werden mittels Autoradiographie nachgewiesen.

Partiell homologe oder heterologe Gene, die verwandt, aber nicht identisch sind, lassen sich analog zum oben beschriebenen Verfahren unter Verwendung niedrig-stringenter Hybridisierungs- und Waschbedingungen identifizieren. Für die wässrige Hybridisierung wurde die Ionenstärke gewöhnlich bei 1 M NaCl gehalten, wobei die Temperatur nach und nach von 68 auf 42°C gesenkt wurde.

Die Isolierung von Gensequenzen, die nur zu einer einzelnen Domäne von beispielsweise 10 bis 20 Aminosäuren Homologien aufweisen, lässt sich unter Verwendung synthetischer, radioaktiv markierter Oligonukleotidsonden durchführen. Radioaktiv markierte Oligonukleotide werden mittels Phosphorylierung des 5'-Endes zweier komplementärer Oligonukleotide mit T4-Polynukleotidkinase hergestellt. Die komplementären Oligonukleotide werden aneinander hybridisiert und ligiert, so dass Konkatemere entstehen. Die doppelsträngigen Konkatemere werden beispielsweise durch Nicktranskription radioaktiv markiert. Die Hybridisierung erfolgt gewöhnlich bei niedrig-stringenten Bedingungen unter Verwendung hoher Oligonukleotidkonzentrationen.

Oligonukleotid-Hybridisierungslösung:

6 x SSC
0,01 M Natriumphosphat
1 mM EDTA (pH 8)
0,5 % SDS
100 mikrog/ml denaturierte Lachssperma-DNA
0,1 % fettarme Trockenmilch

Während der Hybridisierung wird die Temperatur schrittweise auf 5 bis 10°C unter die berechnete Oligonukleotid-Tm oder bis auf Raumtemperatur (bedeutet RT = ~ 23°C in allen Experimenten, wenn nicht anders angegeben) gesenkt, gefolgt von Waschschritten und Autoradiographie. Das Waschen wird mit extrem niedriger Stringenz durchgeführt, zum Beispiel 3 Waschschritte unter Verwendung von 4 X SSC. Weitere Einzelheiten sind wie von Sambrook, J., et al. (1989), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, oder Ausubel, F.M., et al. (1994) "Current Protocols in Molecular Biology", John Wiley & Sons, beschrieben.

Beispiel 8: Identifikation von Zielgenen durch Sichtung von Expressionsbanken mit Antikörpern

Es wurden cDNA-Sequenzen zur Herstellung von rekombinantem Protein zum Beispiel in E. coli verwendet (z.B. Qiagen QIAexpress pQE-System). Die rekombinanten Proteine wurden dann gewöhnlich über Ni-NTA-Affinitätschromatographie (Qiagen) affinitätsgereinigt. Die rekombinanten Proteine wurden dann zur Herstellung spezifischer Antikörper beispielsweise unter Verwendung von Standardtechniken zur Immunisierung von Kaninchen verwendet. Anschließend wurden die Antikörper dann unter Verwendung einer Ni-NTA-Säule, die mit rekombinantem Antigen vorgesättigt wird, affinitätsgereinigt, wie von Gu et al., (1994) BioTechniques 17:257-262 beschrieben. Der Antikörper kann dann zur Durchmusterung von Expressions-cDNA-Banken mittels immunologischem Sichtung verwendet werden (Sambrook, J., et al. (1989), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, oder Ausubel, F.M., et al. (1994) "Current Protocols in Molecular Biology", John Wiley & Sons).

Beispiel 9: Transformation von Agrobacterium

Die Agrobacterium-vermittelte Pflanzentransformation kann zum Beispiel unter Verwendung des GV3101- (pMP90-) (Koncz und Schell, Mol. Gen. Genet. 204 (1986) 383-396) oder LBA4404- (Clontech) oder C58C1 pGV2260 (Deblaere et al 1984, Nucl. Acids Res. 13, 4777-4788) Agrobacterium tumefaciens-Stamms durchgeführt werden. Die Transformation kann durch Standard-Transformationstechniken durchgeführt werden (ebenfalls Deblaere et al. 1984).

Beispiel 10: Pflanzentransformation

Die Agrobacterium-vermittelte Pflanzentransformation kann unter Verwendung von Standard-Transformations- und Regenerationstechniken durchgeführt werden (Gelvin, Stanton B., Schilperoort, Robert A., Plant Molecular Biology Manual, 2. Aufl., Dordrecht: Kluwer Academic Publ., 1995, in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, Bernard R., Thompson, John E., Methods in Plant Molecular Biology and Biotechnology, Boca Raton: CRC Press, 1993, 360 S., ISBN 0-8493-5164-2).

Beispielsweise kann Raps mittels Kotyledonen- oder Hypokotyltransformation transformiert werden (Moloney et al., Plant Cell 8 (1989) 238-242; De Block et al., Plant Physiol. 91 (1989) 694-701). Die Verwendung von Antibiotika für die Agrobacterium- und Pflanzenselektion hängt von dem für die Transformation verwendeten binären Vektor und Agrobacterium-Stamm ab. Die

Rapsselektion wird gewöhnlich unter Verwendung von Kanamycin als selektierbarem Pflanzenmarker durchgeführt.

Der Agrobacterium-vermittelte Gentransfer in Lein (Linum usitatissimum) lässt sich unter Verwendung von beispielsweise einer von Mlynarova et al. (1994) Plant Cell Report 13:282-285 beschriebenen Technik durchführen.

Die Transformation von Soja kann unter Verwendung von beispielsweise einer in EP-A-0 0424 047 (Pioneer Hi-Bred International) oder in EP-A-0 0397 687, US 5,376,543, US 5,169,770 (University Toledo) beschriebenen Technik durchgeführt werden.

Die Pflanzentransformation unter Verwendung von Teilchenbeschuss, Polyethylenglycol-vermittelter DNA-Aufnahme oder über die Siliziumcarbonatfaser-Technik ist beispielsweise beschrieben von Freeling und Walbot "The maize handbook" (1993) ISBN 3-540-97826-7, Springer Verlag New York).

Beispiel 11: Plasmide für die Pflanzentransformation

Zur Pflanzentransformation können binäre Vektoren, wie pBinAR (Höfgen und Willmitzer, Plant Science 66 (1990) 221-230) oder pGPTV (Becker et al 1992, Plant Mol. Biol. 20:1195-1197) oder Derivate davon verwendet werden. Die Konstruktion der binären Vektoren kann durch Ligation der cDNA in Sense- oder Antisense-Orientierung in T-DNA erfolgen. 5' der cDNA aktiviert ein Pflanzenpromotor die Transkription der cDNA. Eine Polyadenylierungssequenz befindet sich 3' von der cDNA. Die binären Vektoren können unterschiedliche Markergene tragen. Insbesondere kann das nptII-Markergen codierend für Kanamycin-Resistenz vermittelt durch Neomycinphosphotransferase gegen die herbizidresistente Form eines Acetolactat Synthasegens (Abkürzung: AHAS oder ALS) ausgetauscht werden. Das ALS-Gen ist beschrieben in Ott et al., J. Mol. Biol. 1996, 263:359-360. Der v-ATPase-c1-Promotor kann in das Plasmid pBin19 oder pGPTV kloniert werden und durch Klonierung vor das ALS Codierregion für die Markergenexpression genutzt werden. Der genannte Promotor entspricht einem 1153 Basenpaarfragment aus beta-Vulgaris (Plant Mol Biol, 1999, 39:463-475). Dabei können sowohl Sulphonylharnstoffe als auch Imidazolinone wie Imazethapyr oder Sulphonylharnstoffe als Antimetaboliten zur Selektion verwendet werden.

Die gewebespezifische Expression lässt sich unter Verwendung eines gewebespezifischen Promotors erzielen. Beispielsweise kann die samenspezifische Expression erreicht werden, indem der DC3- oder der LeB4- oder der USP-Promotor oder der Phaseolin-Promotor 5' der cDNA einkloniert wird. Auch jedes andere samenspezifische Promotorelement wie z.B. der Napin- oder Arcelin Promotor Goossens et al. 1999, Plant Phys. 120(4):1095-1103 und Gerhardt et al. 2000, Biochimica et Biophysica Acta 1490(1-2):87-98) kann verwendet werden. Zur konstitutiven Expression in der ganzen Pflanzen lässt sich der CaMV-35S-Promotor oder ein v-ATPase C1 Promotor verwenden.

Insbesondere lassen sich Gene codierend für Desaturasen und Elongasen durch Konstruktion mehrerer Expressionskassetten hintereinander in einen binären Vektor klonieren, um den Stoffwechselweg in Pflanzen nachzubilden.

Innerhalb einer Expressionskassette kann das zu exprimierende Protein unter Verwendung eines Signalpeptids, beispielsweise für Plastiden, Mitochondrien oder das Endoplasmatische Retikulum, in ein zelluläres Kompartiment dirigiert werden (Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423). Das Signalpeptid wird 5' im Leseraster mit der cDNA einkloniert, um die subzelluläre Lokalisierung des Fusionsprotein zu erreichen.

Beispiele für Multiexpressionskassetten sind im folgenden gegeben.

I.) Promotor-Terminator-Kassetten

Expressionskassetten bestehen aus wenigstens zwei funktionellen Einheiten wie einem Promotor und einem Terminator. Zwischen Promotor und Terminator können weitere gewünschte Gensequenzen wie Targetting-Sequenzen, Codierregionen von Genen oder Teilen davon etc. eingefügt werden. Zum Aufbau von Expressionskassetten werden Promotoren und Terminatoren (USP Promotor: Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67); OCS Terminator: Gielen et al. EMBO J. 3 (1984) 835ff.) mithilfe der Polymerase-kettenreaktion isoliert und mit flankierenden Sequenzen nach Wahl auf Basis von synthetischen Oligonukleotiden maßgeschneidert.

Folgende Oligonukleotide können beispielsweise verwendet werden:
USP1 vorne: CCGGAATTCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA
USP2 vorne: CCGGAATTCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA
USP3 vorne: CCGGAATTCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA
USP1 hinten:AAAACTGCAGGCGGCCGCCCACCGCGGTGGGCTGGCTATGAAGAAATT
USP2 hinten:CGCGGATCCGCTGGCTATGAAGAAATT
USP3 hinten:TCCCCCGGGATCGATGCCGGCAGATCTGCTGGCTATGAAGAAATT
OCS1 vorne: AAAACTGCAGTCTAGAAGGCCTCCTGCTTTAATGAGATAT
OCS2 vorne: CGCGGATCCGATATCGGGCCCGCTAGCGTTAACCCTGCTTTAATGAGATAT
OCS3 vorne: TCCCCCGGGCCATGGCCTGCTTTAATGAGATAT
OCS1 hinten:CCCAAGCTTGGCGCGCCGAGCTCGAATTCGTCGACGGACAATCAGTAAATTGA
OCS2 hinten:CCCAAGCTTGGCGCGCCGAGCTCGAATTCGTCGACGGACAATCAGTAAATTGA
OCS3 hinten:CCCAAGCTTGGCGCGCCGAGCTCGTCGACGGACAATCAGTAAATTGA

Die Methoden sind dem Fachmann auf dem Gebiet bekannt und sind allgemein literaturbekannt.

In einem ersten Schritt werden ein Promotor und ein Terminator über PCR amplifiziert. Dann wird der Terminator in ein Empfängerplasmid kloniert und in einem zweiten Schritt der Promotor vor den Terminator inseriert. Mithin erhält man eine Expressionskassette auf einem Trägerplasmid. Auf Basis des Plamides pUC19 werden die Plasmide pUT1, pUT2 und pUT3 erstellt.

Die Konstrukte sind erfindungsgemäß in SEQ ID NO: 13, 14 und 15 definiert. Sie enthalten auf Basis von pUC19 den USP-Promotor und den OCS Terminator. Auf Basis dieser Plasmide wird das Konstrukt pUT12 erstellt, indem pUT1 mittels SalI/ScaI geschnitten wird und pUT2 mittels XhoI/ScaI geschnitten wird. Die die Expressionskassetten enthaltenden Fragmente werden ligiert und in E. coli XLI blue MRF transformiert. Es wird nach Vereinzelung ampicillinresistenter Kolonien DNA präpariert und per Restriktionsanalyse solche Klone identifiziert, die zwei Expressionskassetten enthalten. Die XhoI/SalI Ligation kompatibler Enden hat dabei die beiden Schnittstellen XhoI und SalI zwischen den Expressionskassetten eliminiert. Es resultiert das Plasmid pUT12, das in SEQ ID NO: 16 definiert ist. Anschließend wird pUT12 wiederum mittels Sal/ScaI geschnitten und pUT3 mittels XhoI/ScaI geschnitten. Die die Expressionskassetten enthaltenden Fragmente werden ligiert und in E. coli XLI blue MRF transformiert. Es wird nach Vereinzelung ampicillinresistenter Kolonien DNA präpariert und per Restriktionsanalyse solche Klone identifiziert, die drei Expressionskassetten enthalten. Auf diese Weise wird ein Set von Multiexpressionskassetten geschaffen, dass für die Insertion gewünschter DNA genutzt werden kann und in Tabelle 3 beschrieben wird und zudem noch weitere Expressionskassetten aufnehmen kann.

Diese enthalten folgende Elemente:

Tabelle 3

pUC19–Derivat	Schnittstellen vor dem USP Promotor	Multiple Klonierungs–Schnittstellen	Schnittstellen hinter dem OCS–Terminator
pUT1	EcoRI/AscI/ SacI/XhoI	BstXI/NotI/ PstI/XbaI/StuI	SalI/EcoRI/ SacI/AscI/ HindIII
pUT2	EcoRI/AscI/ SacI/XhoI	BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/ SacI/AscI/ HindIII
pUT3	EcoRI/AscI/ SacI/XhoI	BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/ AscI/HindIII
pUT12 Doppel–expressionskassette	EcoRI/AscI/ SacI/XhoI	BstXI/NotI/ PstI/XbaI/StuI Und BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/ SacI/AscI/ HindIII
pUT123 Tripel–expressionskassette	EcoRI/AscI/ SacI/XhoI	1.BstXI/NotI/ PstI/XbaI/StuI und 2.BamHI/EcoRV/ ApaI/NheI/ HpaI und 3.BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/HindIII

Weiterhin lassen sich wie beschrieben und wie in Tabelle 4 näher spezifiziert weitere Multiexpressionskassetten mithilfe des

i) USP-Promotors oder mithilfe des
ii) ca. 700 Basenpaare 3'-Fragmentes des LeB4-Promotors oder mithilfe des
iii) DC3-Promotors erzeugen und für samenspezifische Genexpression einsetzen.

Der DC3-Promotor ist beschrieben bei Thomas, Plant Cell 1996, 263:359-368 und besteht lediglich aus der Region -117 bis +27 weshalb er mithin einer der kleinsten bekannten samenspezifischen Promotoren darstellt. Die Expressionskassetten können mehrfach den selben Promotor enthalten oder aber über drei verschiedene Promotoren aufgebaut werden.

Tabelle 4: Multiple Expressionskassetten

Plasmidname des pUC19-Derivates	Schnittstellen vor dem jeweiligen Promotor	Multiple Klonierungs-Schnittstellen	Schnittstellen hinter dem OCS-Terminator
pUT1 (pUC19 mit USP-OCS1)	EcoRI/AscI/SacI/XhoI	(1) BstXI/NotI/PstI/ XbaI/StuI	SalI/EcoRI/SacI/AscI/ HindIII
pDCT (pUC19 mit DC3-OCS)	EcoRI/AscI/SacI/XhoI	(2) BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/SacI/AscI/ HindIII
pLeBT (pUC19-mit LeB4(700)-OCS)	EcoRI/AscI/SacI/XhoI	(3) BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/HindIII
pUD12 (pUC 19 mit mit USP-OCS1 und mit DC3-OCS)	EcoRI/AscI/SacI/XhoI	(1) BstXI/NotI/ PstI/XbaI/StuI und (2) BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/SacI/AscI/ HindIII
pUDL123 Triple expression cassette (pUC19 mit USP/ DC3 und LeB4-700)	EcoRI/AscI/SacI/XhoI	(1) BstXI/NotI/ PstI/XbaI/StuI und (2) BamHI/ (EcoRV*)/ApaI/ NheI/HpaI und (3) BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/HindIII

* EcoRV Schnittstelle schneidet im 700 Basenpaarfragment des LeB4 Promotors (LeB4-700)

Analog lassen sich weitere Promotoren für Multigenkonstrukte erzeugen insbesondere unter Verwendung des

a) 2,7 kB Fragmentes des LeB4-Promotors oder mithilfe des
b) Phaseolin-Promotors oder mithilfe des
c) konstitutiven v-ATPase c1-Promotors.

Es kann insbesondere wünschenswert sein, weitere besonders geeignete Promotoren zum Aufbau samenspezifischer Multiexpressionskassetten wie z.B. den Napin-Promotor oder den Arcelin-5 Promotor zu verwenden.

ii) Erstellung von Expressionskonstrukten in pUC19- oder pGPTV Derivaten, die Promotor und Terminator erhalten und in Kombination mit gewünschten Gensequenzen zur PUFA Genexpression in pflanzlichen Expressionskassetten enthalten.

Multiexpressionskassetten können mittels AscI direkt von pUC19-Derivaten aus Tabelle 3 in den Vektor pGPTV+AscI (siehe iii.)) über die AscI Schnittstelle inseriert werden und stehen zur Inserierung von Zielgenen zur Verfügung. Die entsprechenden Genkonstrukte (pBUT1 ist in SEQUENZ ID NO: 20, pBUT2 ist in

SEQUENZ ID NO: 21, pBUT 3 ist in SEQUENZ ID NO: 22, pBUT12 ist in SEQUENZ ID NO: 22 und pBUT123 ist in SEQUENZ ID NO: 24 dargestellt) stehen erfindungsgemäß als Kit zur Verfügung. Alternativ können Gensequenzen in die pUC19 basierten Expressionskassetten inseriert werden und als AscI Fragment in pGPTV+AscI eingesetzt werden.

In pUT12 wird zunächst über BstXI und XbaI die D-6-Elongase Pp_PSE1 in die erste Kassette inseriert. Dann wird die D-6-Desaturase aus Moos (Pp_des6) über BamHI/NaeI in die zweite Kassette inseriert. Es entsteht das Konstrukt pUT-ED.Das AscI Fragment aus dem Plasmid pUT-ED wird in den mit AscI geschnittenen Vektor pGPTV+AscI inseriert und die Orientierung des inserierten Fragmentes mittels Restriktion oder Sequenzierung ermittelt. Es entsteht das Plasmid pB-DHGLA, dessen vollständige Sequenz in SEQUENZ ID NO. 25 dargestellt ist. Die Codierregion der Physcomitrella delta 6 Elongase ist in SEQUENZ ID NO. 26 dargestellt, die der delta 6 Desaturase aus Physcomitrella in SEQUENZ ID NO: 27.

In pUT123 wird zunächst über BstXI und XbaI die Δ-6-Elongase Pp_PSE1 in die erste Kassette inseriert. Dann wird die Δ-6-Desaturase aus Moos (Pp_des6) über BamHI/NaeI in die zweite Kassette inseriert und schließlich die Δ-5-Desaturase aus Phaeodactylum (Pt_des5) über BglII in die dritte Kassette inseriert. Das Dreifachkonstrukt erhält den Namen pARA1. Unter Berücksichtigung sequenzspezifischer Restriktionsschnittstellen können weitere Expressionskassetten gemäß Tabelle 5 mit der Bezeichnung pARA2, pARA3 und pARA4 erstellt werden.

Das AscI Fragment aus dem Plasmid pARA1 wird in den mit AscI geschnittenen Vektor pGPTV+AscI inseriert und die Orientierung des inserierten Fragmentes mittels Restriktion oder Sequenzierung ermittelt. Die vollständige Sequenz des resultierenden Plasmides pBARA1 ist in SEQUENZ ID NO. 28 dargestellt. Die Codierregion der Physcomitrella delta 6 Elongase ist in SEQUENZ ID NO. 29 dargestellt, die der delta 6 Desaturase aus Physcomitrella in SEQUENZ ID NO: 30 und die der delta-5 Desaturase aus Phaeodactylum tricornutum in SEQUENZ ID NO: 31.

Tabelle 5: Kombinationen von Desaturasen und Elongasen

	Gen Plasmid	Δ-6-Desaturase	Δ-5-Desaturase	Δ-6-Elongase
1	PUT-ED	Pp_des6	---	Pp_PSE1
2	pARA1	Pt_des6	Pt_des5	Pp_PSE1
3	pARA2	Pt_des6	Ce_des5	Pp_PSE1
4	pARA3	Pt_des6	Ce_des5	Pp_PSE1
5	pARA4	Ce_des6	Ce_des5	Ce_PSE1
6	PBDHGLA	Pt_des6	---	Pp_PSE1
7	PBARAI	Pt_des6	Pt_des5	Pp_PSE1

Plasmide 1 bis 5 sind pUC Derivate, Plasmide 6 bis 7 sind binäre Pflanzentransformationsvektoren

Pp = Physcomitrella patens, Pt = Phaeodactylum tricornutum
Pp_PSE1 entspricht der Sequenz aus SEQ ID NO: 9.
PSE = PUFA spezifische Δ-6-Elongase
Ce_des5 = Δ-5-Desaturase aus Caenorhabditis elegans (Genbank Acc. Nr. AF078796)
Ce_des6 = Δ-6-Desaturase aus Caenorhabditis elegans elegans (Genbank Acc. Nr. AF031477, Basen 11-1342)
Ce_PSE1 = Δ-6-Elongase aus Caenorhabditis elegans (Genbank Acc. Nr. AF244356, Basen 1-867)

Auch weitere Desaturasen oder Elongasegensequenzen können in Expressionskassetten beschriebener Art inseriert werden wie z.B. Genbank Acc. Nr. AF231981, NM_013402, AF206662, AF268031, AF226273, AF110510 oder AF110509.

iii) Transfer von Expressionskassetten in Vektoren zur Transformation von Agrobakterium tumefaciens und zur Transformation von Pflanzen

Chimäre Genkonstrukte auf Basis der in pUC19 beschriebenen können mittels AscI in den binären Vektor pGPTV inseriert. Die multiple Klonierungssequenz wird zu diesem Zweck um eine AscI Schnittstelle erweitert. Zu diesem Zweck wird der Polylinker als zwei doppelsträngige Oligonukleotide neu synthetisiert, wobei eine zusätzliche AscI DNA Sequenz eingefügt wird. Das Oligonukleotid wird mittels EcoRI und HindIII in den Vektor pGPTV inseriert. Es entsteht das Plasmid pGPTV+AscI. Die notwendigen Kloniertechniken sind dem Fachmann bekannt und können einfach wie in Beispiel 1 beschrieben nachgelesen werden.

Beispiel 12: *In vivo*-Mutagenese

Die in vivo-Mutagenese von Mikroorganismen kann mittels Passage der Plasmid- (oder einer anderen Vektor-) DNA durch E. coli oder andere Mikroorganismen (z.B. Bacillus spp. oder Hefen, wie Saccharomyces cerevisiae), bei denen die Fähigkeiten, die Unversehrtheit ihrer genetischen Information aufrechtzuerhalten, gestört ist, erfolgen. Übliche Mutator-Stämme haben Mutationen in den Genen für das DNA-Reparatursystem (z.B. mutHLS, mutD, mutT usw.; als Literaturstelle siehe Rupp, W.D. (1996) DNA repair mechanisms, in: Escherichia coli and Salmonella, S. 2277-2294, ASM: Washington). Diese Stämme sind dem Fachmann bekannt. Die Verwendung dieser Stämme ist beispielsweise in Greener, A., und Callahan, M. (1994) Strategies 7:32-34, erläutert. Der Transfer mutierter DNA-Moleküle in Pflanzen erfolgt vorzugsweise nach Selektion und Test der Mikrooganismen. Transgene Pflanzen werden nach verschiedenen Beispielen im Beispielteil dieses Dokumentes erzeugt.

Beispiel 13: Untersuchung der Expression eines rekombinanten Genproduktes in einem transformierten Organismus

Die Aktivität eines rekombinanten Genproduktes im transformierten Wirtsorganismus kann auf der Transkriptions- und/oder der Translationsebene gemessen werden.

Ein geeignetes Verfahren zur Bestimmung der Menge an Transkription des Gens (ein Hinweis auf die Menge an RNA, die für die Translation des Genproduktes zur Verfügung steht) ist die Durchführung eines Northern-Blots wie unten ausgeführt (als Bezugsstelle siehe Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York, oder den oben erwähnten Beispielteil), wobei ein Primer, der so gestaltet ist, dass er an das Gen von Interesse bindet, mit einer nachweisbaren Markierung (gewöhnlich radioaktiv oder chemilumineszent) markiert wird, so dass, wenn die Gesamt-RNA einer Kultur des Organismus extrahiert, auf einem Gel aufgetrennt, auf eine stabile Matrix transferiert und mit dieser Sonde inkubiert wird, die Bindung und das Ausmaß der Bindung der Sonde das Vorliegen und auch die Menge der mRNA für dieses Gen anzeigt. Diese Information zeigt den Grad der Transkription des transformierten Gens an. Zelluläre Gesamt-RNA kann aus Zellen, Geweben oder Organen mit mehreren Verfahren, die alle im Fachgebiet bekannt sind, wie zum Beispiel das von Bormann, E.R., et al. (1992) Mol. Microbiol. 6:317-326 beschriebene, präpariert werden.

Northern-Hybridisierung

Für die RNA-Hybridisierung wurden 20 µg Gesamt-RNA oder 1 µg poly(A)$^+$-RNA mittels Gelelektrophorese in Agarosegelen mit einer Stärke von 1,25 % unter Verwendung von Formaldehyd, wie beschrieben in Amasino (1986, Anal. Biochem. 152, 304) aufgetrennt, mittels Kapillaranziehung unter Verwendung von 10 x SSC auf positiv geladene Nylonmembranen (Hybond N+, Amersham, Braunschweig) übertragen, mittels UV-Licht immobilisiert und 3 Stunden bei 68°C unter Verwendung von Hybridisierungspuffer (10 % Dextransulfat Gew./Vol., 1 M NaCl, 1 % SDS, 100 mg Heringssperma-DNA) vorhybridisiert. Die Markierung der DNA-Sonde mit dem Highprime DNA labeling-Kit (Roche, Mannheim, Deutschland) erfolgte während der Vorhybridisierung unter Verwendung von alpha-^{32}P-dCTP (Amersham, Braunschweig, Deutschland). Die Hybridisierung wurde nach Zugabe der markierten DNA-Sonde im gleichen Puffer bei 68°C über Nacht durchgeführt. Die Waschschritte wurden zweimal für 15 min unter Verwendung von 2 X SSC und zweimal für 30 min unter Verwendung von 1 X SSC, 1 % SDS, bei 68°C durchgeführt. Die Exposition der verschlossenen Filter wurde bei -70°C für einen Zeitraum von 1 bis 14 T durchgeführt.

Zur Untersuchung des Vorliegens oder der relativen Menge an von dieser mRNA translatiertem Protein können Standardtechniken, wie ein Western-Blot, eingesetzt werden (siehe beispielsweise Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York). Bei diesem Verfahren werden die zellulären Gesamt-Proteine extrahiert, mittels Gelelektrophorese aufgetrennt, auf eine Matrix, wie Nitrozellulose, übertragen und mit einer Sonde, wie einem Antikörper, der spezifisch an das gewünschte Protein bindet, inkubiert. Diese Sonde ist gewöhnlich mit einer chemilumineszenten oder kolorimetrischen Markierung versehen, die sich leicht nachweisen lässt. Das Vorliegen und die Menge der beobachteten Markierung zeigt das Vorliegen und die Menge des gewünschten, in der Zelle vorliegenden mutierten Proteins an.

Beispiel 14: Analyse der Auswirkung der rekombinanten Proteine auf die Produktion des gewünschten Produktes

Die Auswirkung der genetischen Modifikation in Pflanzen, Pilzen, Algen, Ciliaten oder auf die Produktion einer gewünschten Verbindung (wie einer Fettsäure) kann bestimmt werden, indem die modifizierten Mikroorganismen oder die modifizierte Pflanze unter geeigneten Bedingungen (wie den vorstehend beschriebenen) gezüchtet werden und das Medium und/oder die zellulären Komponenten auf die erhöhte Produktion des gewünschten Produktes (d.h. von Lipiden oder einer Fettsäure) untersucht wird. Diese Analyse-

techniken sind dem Fachmann bekannt und umfassen Spektroskopie, Dünnschichtchromatographie, Färbeverfahren verschiedener Art, enzymatische und mikrobiologische Verfahren sowie analytische Chromatographie, wie Hochleistungs-Flüssigkeitschromatographie (siehe beispielsweise Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; und Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

Neben den oben erwähnten Verfahren werden Pflanzenlipide aus Pflanzenmaterial wie von Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, und Browse et al. (1986) Analytic Biochemistry 152:141-145, beschrieben extrahiert. Die qualitative und quantitative Lipid- oder Fettsäureanalyse ist beschrieben bei Christie, William W., Advances in Lipid Methodology, Ayr/ Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

Zusätzlich zur Messung des Endproduktes der Fermentation ist es auch möglich, andere Komponenten der Stoffwechselwege zu analysieren, die zur Produktion der gewünschten Verbindung verwendet werden, wie Zwischen- und Nebenprodukte, um die Gesamteffizienz der Produktion der Verbindung zu bestimmen. Die Analyseverfahren umfassen Messungen der Nährstoffmengen im Medium (z.B. Zucker, Kohlenwasserstoffe, Stickstoffquellen, Phosphat und andere Ionen), Messungen der Biomassezusammensetzung und des Wachstums, Analyse der Produktion üblicher Metabolite von Biosynthesewegen und Messungen von Gasen, die während der Fermentation erzeugt werden. Standardverfahren für diese Messungen sind in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes und P.F. Stanbury, Hrsgb., IRL Press, S. 103-129; 131-163 und 165-192

(ISBN: 0199635773) und darin angegebenen Literaturstellen beschrieben.

Ein Beispiel ist die Analyse von Fettsäuren (Abkürzungen: FAME, Fettsäuremethylester; GC-MS, Gas-Flüssigkeitschromatographie-Massenspektrometrie; TAG, Triacylglycerin; TLC, Dünnschichtchromatographie).

Der unzweideutige Nachweis für das Vorliegen von Fettsäureprodukten kann mittels Analyse rekombinanter Organismen nach Standard-Analyseverfahren erhalten werden: GC, GC-MS oder TLC, wie verschiedentlich beschrieben von Christie und den Literaturstellen darin (1997, in: Advances on Lipid Methodology, Vierte Aufl.: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren, Lipide 33:343-353).

Das zu analysierende Material kann durch Ultraschallbehandlung, Mahlen in der Glasmühle, flüssigen Stickstoff und Mahlen oder über andere anwendbare Verfahren aufgebrochen werden. Das Material muss nach dem Aufbrechen zentrifugiert werden. Das Sediment wird in Aqua dest. resuspendiert, 10 min bei 100°C erhitzt, auf Eis abgekühlt und erneut zentrifugiert, gefolgt von Extraktion in 0,5 M Schwefelsäure in Methanol mit 2 % Dimethoxypropan für 1 Std. bei 90°C, was zu hydrolysierten Öl- und Lipidverbindungen führt, die transmethylierte Lipide ergeben. Diese Fettsäuremethylester werden in Petrolether extrahiert und schließlich einer GC-Analyse unter Verwendung einer Kapillarsäule (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 mikrom, 0,32 mm) bei einem Temperaturgradienten zwischen 170°C und 240°C für 20 min und 5 min bei 240°C unterworfen. Die Identität der erhaltenen Fettsäuremethylester muss unter Verwendung von Standards, die aus kommerziellen Quellen erhältlich sind (d.h. Sigma), definiert werden.

Bei Fettsäuren, für die keine Standards verfügbar sind, muss die Identität über Derivatisierung und anschließende GC-MS-Analyse gezeigt werden. Beispielsweise muss die Lokalisierung von Fettsäuren mit Dreifachbindung über GC-MS nach Derivatisierung mit 4,4-Dimethoxyoxazolin-Derivaten (Christie, 1998, siehe oben) gezeigt werden.

Expressionskonstrukte in heterologen mikrobiellen Systemen

Stämme, Wachstumsbedingungen und Plasmide

Der Escherichia coli-Stamm XL1 Blue MRF' kan (Stratagene) wurde zur Subklonierung der neuen Desaturase pPDesaturase1 aus Physcomitrella patens verwendet. Für die funktionelle Expression dieses Gens verwendeten wir den Saccharomyces cerevisiae-Stamm INVSc 1 (Invitrogen Co.). E. coli wurde in Luria-Bertini-Brühe (LB, Duchefa, Haarlem, Niederlande) bei 37°C kultiviert. Wenn nötig, wurde Ampicillin (100 mg/Liter) zugegeben, und 1,5 % Agar (Gew./Vol.) wurde für feste LB-Medien hinzugefügt. S. cerevisiae wurde bei 30°C entweder in YPG-Medium oder in komplettem Minimalmedium ohne Uracil (CMdum; siehe in: Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., Albright, L.B., Coen, D.M., und Varki, A. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York) mit entweder 2 % (Gew./Vol.) Raffinose oder Glucose kultiviert. Für feste Medien wurden 2 % (Gew./Vol.) Bacto™-Agar (Difco) hinzugefügt. Die zur Klonierung und Expression verwendeten Plasmide sind pUC18 (Pharmacia) und pYES2 (Invitrogen Co.).

Beispiel 16: Klonierung und Expression PUFA-spezifischer Desaturasen aus Phaeodactylum tricornutum

Für die Expression in Hefe wurden die Phaeodactylum tricornutum -cDNA-Klone aus Seq ID NO: 1, 3, 5 oder 11 bzw. die Sequenzen aus SEQ ID NO: 7 oder 9 bzw andere gewünschte Sequenzen zuerst so modifiziert, dass lediglich die Codierregion mittels Polymerase Kettenreaktion unter Zuhilfenahme zweier Oligonukleotide amplifiziert werden. Dabei wurde darauf geachtet, dass eine Konsensussequenz vor dem Startcodon zur effizienten Translation eingehalten wurde. Entweder wurde hierzu die Basenfolge ATA oder AAA gewählt und vor das ATG in die Sequenz eingefügt (Kozak, M. (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes, Cell 44, 283-292). Vor diesem Konsensustriplett wurde zusätzlich eine Restriktionsschnittstelle eingeführt, die kompatibel sein muss zur Schnittstelle des Zielvektors, in den das Fragment kloniert werden soll und mit dessen Hilfe die Genexpression in Mikroorganismen oder Pflanzen erfolgen soll.

Die PCR-Reaktion wurde mit Plasmid-DNA als Matrize in einem Thermocycler (Biometra) mit der Pfu-DNA-(Stratagene)Polymerase und dem folgenden Temperaturprogramm durchgeführt: 3 min bei 96°C, gefolgt von 30 Zyklen mit 30 s bei 96°C, 30 s bei 55°C und 2 min bei 72°C, 1 Zyklus mit 10 min bei 72°C und Stop bei 4°C. Die Anlagerungstemperatur wurde je nach gewählten Oligonukleotiden variiert. Pro Kilobasenpaare DNA ist von einer Synthesezeit von etwa einer Minute auszugehen. Weitere Parameter, die Einfluss auf die PCR haben wie z.B. Mg-Ionen, Salz, DNA Polymerase etc., sind dem Fachmann auf dem Gebiet geläufig und können nach Bedarf variiert werden.

Die korrekte Größe des amplifizierten DNA-Fragments wurde mittels Agarose-TBE-Gelelektrophorese bestätigt. Die amplifizierte DNA wurde aus dem Gel mit dem QIAquick-Gelextraktionskit (QIAGEN) extrahiert und in die SmaI-Restriktionsstelle des dephosphorylierten Vektors pUC18 unter Verwendung des Sure Clone Ligations Kit (Pharmacia) ligiert, wobei die pUC-Derivate erhalten wurden. Nach der Transformation von E. coli XL1 Blue MRF' kan wurde eine DNA-Minipräparation (Riggs, M.G., & McLachlan, A. (1986) A simplified screening procedure for large numbers of plasmid mini-preparation. BioTechniques 4, 310-313) an ampicillinresistenten Transformanden durchgeführt, und positive Klone mittels BamHI-Restriktionsanalyse identifiziert. Die Sequenz des klonierten PCR-Produktes wurde mittels Resequenzierung unter Verwendung des ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Weiterstadt) bestätigt.

Δ5 Acyl Lipid desaturase, Pt_des5
Primer 1 GAG CTC ACA TAA TGG CTC CGG ATG CGG ATA AGC
Primer 2 CTC GAG TTA CGC CCG TCC GGT CAA GGG

Das PCR-Fragment (1428bp) wurde mithilfe des Sure Clone Kit (Pharmacia) in pUC 18 kloniert, das inserierte Fragment SacI/XhoI verdaut und das Fragment mithilfe entsprechender Restriktionsschnittstellen in pYES2 oder pYES6 inseriert.

Δ6 Acyl Lipid desaturase, Pt_des6
Primer 3 GGA TCC ACA TAA TGG GCA AAG GAG GGG ACG CTC GGG
Primer 4 CTC GAG TTA CAT GGC GGG TCC ATC GGG

Das PCR-Fragment (1451 bp) wurde mithilfe des Sure Clone Kit (Pharmacia) in pUC 18 kloniert, das inserierte Fragment BamHI/XhoI verdaut und das Fragment mithilfe entsprechender Restriktionsschnittstellen in pYES2 oder pYES6 inseriert.

Δ12 Acyl Lipid desaturase, Pt_des12
Primer 5 GGA TCC ACA TAA TGG TTC GCT TTT CAA CAG CC
Primer 6 CTC GAG TTA TTC GCT CGA TAA TTT GC

Δ12 Acyl Lipid desaturase, Pt_des12.2
Primer 7 GGA TCC ACA TAA TGG GTA AGG GAG GTC AAC G
Primer 8 CTC GAG TCA TGC GGC TTT GTT TCG C

Das PCR Fragment (1505bp) wurde mithilfe des Sure Clone Kit (Pharmacia) in pUC 18 kloniert, das inserierte Fragment BamHI/XhoI verdaut und das Fragment mithilfe entsprechender Restriktionsschnittstellen in pYES2 oder pYES6 inseriert.

Die Plasmid-DNA wurde mit Restriktionsenzym/en passend zur eingeführten Schnittstelle der Primersequenz gespalten und das erhaltene Fragment in die kompatiblen Restriktionsstellen des dephosphorylierten Hefe-E. coli-Shuttlevektors pYES2 oder pYES6 ligiert, wobei pYES-Derivate erhalten werden. Nach der Transformation von E. coli und DNA-Minipräparation aus den Transformanden wurde die Orientierung des DNA-Fragments im Vektor durch geeignete Restriktionsspaltung oder Sequenzierung überprüft. Ein Klon wurde für die DNA-Maxipräparation mit dem Nucleobond® AX 500 Plasmid-DNA-Extraktionskit (Macherey-Nagel, Düringen) angezogen.

Saccharomyces cerevisiae INVSc1 wurde mit den pYES-Derivaten und pYES Leervektor mittels eines PEG/Lithiumacetat-Protokolls transformiert (Ausubel et al., 1995). Nach der Selektion auf CMdum-Agarplatten mit 2 % Glucose wurden pYES-Derivate-Transformanden und eine pYES2-Transformande zur weiteren Anzucht und funktionellen Expression ausgewählt. Bei pYES6-Derivaten wurde Blasticidin als Antimetabolit verwendet. Im Fall von Coexpressionen auf Basis von pYES2 und pYES6 wurde auf Minimalmedium mit Blasticidin selektiert.

Funktionelle Expression einer Desaturaseaktivität in Hefe

Vorkultur

20 ml CMdum-Flüssigmedium ohne Uracil aber mit 2 % (Gew./Vol.) Raffinose wurden mit den transgenen Hefeklonen (pYES2) angeimpft und 3 Tage bei 30°C, 200 rpm gezüchtet, bis eine optische Dichte bei 600 nm (OD_{600}) von 1,5 bis 2 erreicht wurde. Wurde als Vektor pYES6 verwendet, so wurde zusätzlich auf Blasticidin als Antimetabolit selektioniert.

Hauptkultur

Für die Expression wurden 20 ml CMdum-Flüssigmedium ohne Uracil aber mit 2 % Raffinose und 1 % (Vol./Vol.) Tergitol NP-40 mit Fettsäuresubstraten auf eine Endkonzentration von 0,003 % (Gew./Vol.) angereichert. Die Medien wurden mit den Vorkulturen auf eine OD_{600} von 0,05 angeimpft. Die Expression wurde bei einer OD_{600} von 0,2 mit 2 % (Gew./Vol.) Galaktose für 16 Std. induziert, wonach die Kulturen eine OD_{600} von 0,8-1,2 geerntet wurden.

Fettsäureanalyse

Die Gesamt-Fettsäuren wurden aus Hefekulturen extrahiert und mittels Gaschromatographie analysiert. Davon wurden Zellen von 5 ml Kultur mittels Zentrifugation (1000 x g, 10 min, 4°C) geerntet und einmal mit 100 mM $NaHCO_3$, pH 8,0, gewaschen, um restliches Medium und Fettsäuren zu entfernen. Zur Herstellung des Fettsäuremethylester (FAMES oder Singular FAME) wurden die Zellsedimente mit 1 M methanolischer H_2SO_4 und 2 % (Vol./Vol.) Dimethoxypropan für 1 Std. bei 80°C behandelt. Die FAMES wurden zweimal mit 2 ml Petrolether extrahiert, einmal mit 100 mM $NaHCO_3$, pH 8,0, und einmal mit destilliertem Wasser gewaschen und mit Na_2SO_4 getrocknet. Das organische Lösungsmittel wurde unter einem Argonstrom verdampft, und die FAMES wurden in 50 mikrol Petrolether gelöst. Die Proben wurden auf einer ZEBRON-ZB-Wax-Kapillarsäule (30 m, 0,32 mm, 0,25 mikro m; Phenomenex) in einem Hewlett Packard-6850-Gaschromatograph mit einem Flammenionisationsdetektor aufgetrennt. Die Ofentemperatur wurde von 70°C (1 min halten) bis 200°C mit einer Rate von 20°C/min, dann auf 250°C (5 min halten) mit einer Rate von 5°C/min und schließlich auf 260°C mit einer Rate von 5°C/min programmiert. Stickstoff wurde als Trägergas verwendet (4,5 ml/min bei 70°C). Die Fettsäuren wurden durch Vergleich mit Retentionszeiten von FAME-Standards (SIGMA) identifiziert.

Expressionsanalyse

Die Verhältnisse der zugegebenen und aufgenommenen Fettsäuresubstrate wurden ermittelt und so Quantität und Qualität der Desaturasereaktion gemäß Tabelle 6, Tabelle 7 und Tabelle 8 erfasst.

Ergebnis der Expression einer Phaeodactylum tricornutum Δ-6-Acyl Lipid Desaturase in Hefe:

Tabelle 6

Fettsäure	pYes2	pYes2-Ptd6 gefüttert mit		
	-	-	+18:2	+18:3
16:0	13,3	18,9	28,4	16,7
16:1Δ9	45,4	44,7	12,5	16,9
16:2Δ6,9	-	4,3	-	-
18:0	4,9	6,3	10,4	9,1
18:1Δ9	36,4	24,1	6,8	11,8
18:2Δ6,9	-	1,8	-	-
18:2Δ9,12	-	-	33,4	-
18:3Δ6,12,15	-	-	4,9	-
18:3Δ9,12,15	-	-		43,1
18:4Δ6,9,12,15	-	-	-	2,3

Die Angaben stellen Mol-% entsprechender cis-Fettsäuren dar.

Ergebnis der Expression einer Phaeodactylum tricornutum Δ-5-Acyl Lipid Desaturase in Hefe:

Tabelle 7

	pYES2		pYES_PtD5-Konstrukt gefüttert mit						
Fett-säure	Leer	Kon-. trolle	18:2	18:3	20:1 Δ8	-20:1 Δ11	20:2 Δ11,14	20:3 Ω3	20:3 Ω6
16:0Δ	16,9	20,4	27,7	24,4	16,2	21	17,6	19,5	22,8
16:1Δ9	44,7	44,1	13,2	9,6	37,4	39,4	38,3	36,9	30,7
18:0	6,1	6,9	10,54	9,8	4,7	7,9	6,3	6,8	8,2
18:1 Δ9	31,72	28,1	8,77	6	15	26	29,5	25,6	21,1
18:2 Δ5,9		0,17	0	0	0	0,09	0,21	0,09	9
18:2 Δ9,12		-	39,7	-	-	-	-	-	-
18:3 Δ9,12,15		-		49,9	--	-	-	-	-
20:1 Δ8		-		-	25,5	-	-	-	-
20:1 Δ11		-		-	-	5,41	-	-	-
20:2 Δ5,11		-		-	-	0,21	-	-	-
20:2 Δ11,14		-	-	-	-	-	6,48	-	-
20:3 Δ5,11,14		-					0,76	-	-
20:3 Δ11,14,17		-	-	-	-	-	-	9,83	-
20:3 Δ8,11,14		-	-	-	-	-	-	-	13,69
20:4 Δ5,11,14,17		-	-	-	-	-	-	1,16	-
20:4 Δ5,8,11,14		-	-	-	-	-	-	-	3,08

Die Angaben stellen Mol-% Fettsäuren von cis-Fettsäuren dar.

Aus weiteren Fütterungsversuchen wurde gefunden, dass C18:1Δ9 in der Anwesenheit von C18:2 Δ9,11 oder C18:3 Δ9,12,15 oder C20:1 Δ8 Fettsäuren nicht desaturiert wurde während in Anwesenheit von C20:1 Δ11, C20:2 Δ11,14 und C20:3 Δ8,11,14 auch C18:1 desaturiert wird. Ebenfalls keine Desaturierung erfolgte in Anwesenheit von C20:3 Δ8,11,14.

Bei Nutzung des Protease-defizienten Hefestammes C13BYS86 (Kunze I. et al., Biochemica et Biophysica Acta (1999) 1410:287-298) für die Expression der Δ-5-Desaturase aus Phaeodactylum tricornutum auf Vollmedium mit Blasticidin wurde gefunden, dass C20:4 Δ8,11,14,17 als Substrat der Δ-5-Desaturase mit 20 % Umsatzrate ebenso gut umgesetzt wurde wie C20:3 Δ8,11,14. Alternativ können auch die Auxotrophiemarker leu2, ura3 oder his für Genexpression genutzt werden.

In einem weiteren Coexpressionsexperiment von Δ-5 Desaturase aus Phaeodactylum und Δ-6 Elongase aus Physcomitrella wurde der Stamm UTL7A (Warnecke et al., J. Biol. Chem. (1999) 274(19):13048-13059)benutzt, wobei die Δ-5 Desaturase ca 10 % C20:3 Δ8,11,14 zu C20:4 Δ5,8,11,14 umsetzte.

Weitere Fütterungsexperimente mit verschiedensten anderen Fettsäuren allein oder in Kombination (z.B. Linolsäure, 20:3 Δ-5,11,14-Fettsäure, alpha- oder gamma Linolensäure, Stearidonsäure, Arachidonsäure, Eicosapentaensäure etc.) können zur detaillierteren Bestätigung der Substratspezifität und -Selektivität dieser Desaturasen durchgeführt werden.

Tabelle 8: Ergebnis der Coxpression einer Phaeodactylum tricornutum Δ-5-Acyl Lipid Desaturase und einer Δ-6 Elongase aus Moos in Hefe auf Basis der Expressionsvektoren pYes2 und pYes6

	pYes2-Elo		pYes2-Elo and pYes6-Ptd5	
	+18:3	+18:4	+18:3	+18:4
16:0	15,0	14,8	15,6	15,1
16:1Δ9	27,7	29,2	27,5	29,0
18:0	5,6	6,3	5,7	6,4
18:1Δ9	17,1	30,8	27,4	31,6
18:3Δ6,9,12	7,60	-	7,8	-
18:4Δ6,9,12,15	-	6,71	-	6,4
20:3Δ8,11,14	15,92	-	13,55	-
20:4Δ5,8,11,14	-	-	1,31	-
20:4Δ8,11,14,17	-	11,4	-	10,31
20:5Δ5,8,11,14,17	-	-	-	0,53

Aus den Substratumsetzungen geht hervor, dass die verwendete Δ-5-Desaturase aus Phaeodactylum und die Δ-6-Elongase aus Physcomitrella patens bzgl. der Substrataktivität und insbesondere der Substratspezifität geeignet sind, um Arachidonsäure bzw. Eicosapentaensäure mithilfe erfindungsgemäßer Sequenzen zu produzieren.

Die Fragmentierungsmuster und Massenspektren von DMOX-Derivaten von Standards als auch den Peakfraktionen per GC identifizierter Fettsäuren der in Tabelle 6, 7 und 8 aufgeführten, zeigen vergleichsweise identische Ergebnisse, wodurch die jeweilige Position der Doppelbindung über die bloße GC-Detektion hinaus abgesichert wurde.

Beispiel 17: Reinigung des gewünschten Produktes aus transformierten Organismen

Die Gewinnung des gewünschten Produktes aus Pflanzenmaterial oder Pilzen, Algen, Ciliaten, tierischen Zellen oder aus dem Überstand der vorstehend beschriebenen Kulturen kann durch verschiedene, im Fachgebiet bekannte Verfahren erfolgen. Wird das gewünschte Produkt nicht aus den Zellen sezerniert, können die Zellen aus der Kultur durch langsame Zentrifugation geerntet werden, die Zellen können durch Standardtechniken, wie mechanische Kraft oder Ultraschallbehandlung, lysiert werden. Organe von Pflanzen können mechanisch von anderem Gewebe oder anderen Organen getrennt werden. Nach der Homogenisation werden die Zelltrümmer durch Zentrifugation entfernt, und die Überstandsfraktion, welche die löslichen Proteine enthält, wird zur weiteren Reinigung der gewünschten Verbindung aufbewahrt. Wird das Produkt aus gewünschten Zellen sezerniert, werden die Zellen durch langsame Zentrifugation aus der Kultur entfernt, und die Überstandsfraktion wird zur weiteren Reinigung aufbewahrt.

Die Überstandsfraktion aus jedem Reinigungsverfahren wird einer Chromatographie mit einem geeigneten Harz unterworfen, wobei das gewünschte Molekül entweder auf dem Chromatographieharz zurückgehalten wird, viele Verunreinigungen in der Probe jedoch nicht, oder die Verunreinigungen auf dem Harz zurückbleiben, die Probe hingegen nicht. Diese Chromatographieschritte können wenn nötig wiederholt werden, wobei die gleichen oder andere Chromatographieharze verwendet werden. Der Fachmann ist in der Auswahl geeigneter Chromatographieharze und ihrer wirksamsten Anwendung für ein bestimmtes zu reinigendes Molekül bewandert. Das gereinigte Produkt kann durch Filtration oder Ultrafiltration konzentriert und bei einer Temperatur aufbewahrt werden, bei der die Stabilität des Produktes maximal ist.

Im Fachgebiet ist ein breites Spektrum an Reinigungsverfahren bekannt, und das vorstehende Reinigungsverfahren soll nicht beschränkend sein. Diese Reinigungsverfahren sind zum Beispiel beschrieben in Bailey, J.E., & Ollis, D.F., Biochemical Engineering Fundamentals, McGraw-Hill: New York (1986).

Die Identität und Reinheit der isolierten Verbindungen kann durch Standardtechniken des Fachgebiets bestimmt werden. Dazu gehören Hochleistungs-Flüssigkeitschromatographie (HPLC), spektroskopische Verfahren, Färbeverfahren, Dünnschichtchromatographie, insbesondere Dünnschichtchromatographie und Flammenionisationsdetektion (IATROSCAN, Iatron, Tokio, Japan), NIRS, Enzymtest oder mikrobiologisch. Eine Übersicht über diese Analyseverfahren siehe in: Patek et al. (1994) Appl. Environ. Microbiol. 60:133-140; Malakhova et al. (1996) Biotekhnologiya 11:27-32; und Schmidt et al. (1998) Bioprocess Engineer. 19:67-70. Ulmann's Encyclopedia of Industrial Chemistry (1996) Bd. A27, VCH: Weinheim, S. 89-90, S. 521-540, S. 540-547, S. 559-566, 575-581 und S. 581-587; Michal, G (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley and Sons; Fallon, A., et al. (1987) Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17.

Äquivalente

Der Fachmann erkennt oder kann viele Äquivalente der hier beschriebenen erfindungsgemäßen spezifischen Ausführungsformen feststellen, indem er lediglich Routineexperimente verwendet. Diese Äquivalente sollen von den Patentansprüchen umfasst sein.

Patentansprüche

1. Verfahren zur Herstellung von Fettsäureestern mit einem erhöhtem Gehalt an mehrfach ungesättigten Fettsäuren mit mindestens zwei Doppelbindungen dadurch gekennzeichnet, daß man mindestens eine Nukleinsäuresequenz ausgewählt aus der Gruppe

   a) einer Nukleinsäuresequenz mit der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Sequenz,

   b) Nukleinsäuresequenzen, die aufgrund des degenerierten genetischen Codes durch Rückübersetzung der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen erhalten werden,

   c) Derivate der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Nukleinsäuresequenz, die für Polypeptide mit der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen codieren und mindestens 50 % Homologie auf Aminosäureebene aufweisen, ohne daß die enzymatische Wirkung der Polypeptide wesentlich reduziert ist,

   in einen Fettsäureester produzierenden Organismus einbringt, anzieht und die in dem Organismus enthaltenen Fettsäureester isoliert.

2. Verfahren nach Anspruch 1, wobei die durch das Verfahren hergestellten Fettsäureester mehrfach ungesättigte C_{18}-, C_{20}- oder C_{22}-Fettsäuremoleküle mit mindestens zwei Doppelbindungen im Fettsäureester enthalten.

3. Verfahren nach Anspruch 1 oder 2, wobei die C_{18}-, C_{20}- oder C_{22}-Fettsäuremoleküle aus dem Organismus in Form eines Öls, Lipids isoliert werden.

4. Verfahren nach den Ansprüchen 1 bis 3, wobei der Organismus ein Mikroorganismus, ein Tier oder eine Pflanze ist.

Zeichn.

5. Verfahren nach den Ansprüchen 1 bis 4, wobei der Organismus eine transgene Pflanze ist.

6. Verfahren nach den Ansprüchen 1 bis 5, wobei die Fettsäureester C_{18}-, C_{20}- oder C_{22}-Fettsäuren mit drei, vier oder fünf Doppelbindungen im Fettsäureester enthalten.

7. Verfahren nach den Ansprüchen 1 bis 6, dadurch gekennzeichnet, daß man die in den Fettsäureestern enthaltenden mehrfach ungesättigten Fettsäuren freisetzt.

8. Isolierte Nukleinsäuresequenz, die für ein Polypeptid mit Desaturaseaktivität codiert, ausgewählt aus der Gruppe:

a) einer Nukleinsäuresequenz mit der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Sequenz,

b) Nukleinsäuresequenzen, die aufgrund des degenerierten genetischen Codes durch Rückübersetzung der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen erhalten werden,

c) Derivate der in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellten Nukleinsäuresequenz, die für Polypeptide mit der in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 oder SEQ ID NO: 12 dargestellten Aminosäuresequenzen codieren und mindestens 50 % Homologie auf Aminosäureebene aufweisen, ohne daß die enzymatische Wirkung der Polypeptide wesentlich reduziert ist.

9. Aminosäuresequenz codiert durch eine Nukleinsäuresequenz gemäß Anspruch 8.

10. Aminosäuresequenz nach Anspruch 9, codiert durch die in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dargestellte Sequenz.

11. Nukleinsäurekonstrukt enthaltend eine Nukleinsäuresequenz gemäß Anspruch 8, wobei die Nukleinsäuresequenz mit einem oder mehreren Regulationssignalen verknüpft ist.

12. Nukleinsäurekonstrukt nach Anspruch 11, wobei im Nukleinsäurekonstrukt zusätzliche zusätzliche Biosynthesegene des Fettsäure- oder Lipidstoffwechsels enthalten sind.

13. Nukleinsäurekonstrukt nach Anspruch 11 oder 12, wobei als Biosynthesegen des Fettsäure- oder Lipidstoffwechsels im Nukleinsäurekonstrukt ein Gen ausgewählt aus der Gruppe Acyl-CoA-Dehydrogenase(n), Acyl-ACP[= acyl carrier protein]-Desaturase(n), Acyl-ACP-Thioesterase(n), Fettsäure-Acyl-Transferase(n), Fettsäure-Synthase(n), Fettsäure-Hydroxylase(n), Acetyl-Coenzym A-Carboxylase(n), Acyl-Coenzym A-Oxidase(n), Fettsäure-Desaturase(n), Fettsäure-Acetylenasen, Lipoxygenasen, Triacylglycerol-Lipasen, Allenoxid-Synthasen, Hydroperoxid-Lyasen oder Fettsäure-Elongase(n) enthalten ist.

14. Vektor enthaltend eine Nukleinsäuresequenz gemäß Anspruch 8 oder ein Nukleinsäurekonstrukt gemäß Anspruch 11.

15. Organismus enthaltend mindestens eine Nukleinsäuresequenz gemäß Anspruch 8, mindestens ein Nukleinsäurekonstrukt gemäß Anspruch 11 oder mindestens einen Vektor gemäß Anspruch 14.

16. Organismus nach Anspruch 15, wobei es sich bei dem Organismus um eine Pflanze, einen Mikroorganismus oder ein Tier handelt.

17. Transgene Pflanze enthaltend eine funktionelle oder nicht funktionelle Nukleinsäuresequenz gemäß Anspruch 8, ein funktionelles oder nicht funktionelles Nukleinsäurekonstrukt gemäß Anspruch 11 oder einen funktionellen oder nicht funktionellen Vektor gemäß Anspruch 14.

18. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 8 oder eines Nukleinsäurekonstrukt gemäß Anspruch 11 zur Herstellung von transgenen Pflanzen.

19. Antikörper, der spezifisch ein Polypeptid, das von einer der Nukleinsäuren gemäß Anspruch 8 codiert wird, bindet.

20. Antisense-Nukleinsäuremolekül, das die Komplementärsequenz der Nukleinsäure gemäß Anspruch 8 umfasst.

21. Öl, Lipide oder Fettsäuren oder eine Fraktion davon, hergestellt durch das Verfahren gemäß den Ansprüchen 1 bis 7.

22. Öl-, Lipid- oder Fettsäurezusammensetzung, die Öl, Lipide oder Fettsäuren oder eine Fraktion davon gemäß Anspruch 21 enthalten.

23. Verwendung der Öl, Lipide oder Fettsäuren oder eine Fraktion davon gemäß Anspruch 21 oder der Öl-, Lipid- oder Fettsäurezusammensetzung gemäß Anspruch 22 in Futter, Nahrungsmitteln, Kosmetika oder Pharmazeutika.

24. Verfahren zur Identifikation eines Antagonisten oder Agonisten von Desaturasen, umfassend

a) in Kontaktbringen der Zellen, die das Polypeptid der vorliegenden Erfindung exprimieren, mit einem Kandidatenstoff;

b) Testen der Desaturase-Aktivität;

c) Vergleichen der Desaturase-Aktivität mit einer Standardaktivität in Abwesenheit des Kandidatenstoffs, wobei ein Anstieg der Desaturase-Aktivität über den Standard anzeigt, daß der Kandidatenstoff ein Agonist und ein Verringerung der Desaturase-Aktivität anzeigt, daß der Kandidatenstoff ein Antagonist ist.

25. Kit, umfassend die Nukleinsäure gemäß Anspruch 8, das Nukleinsäurekonstrukt nach den Ansprüchen 11 bis 13, den Antikörper gemäß Anspruch 19, das Antisense-Nukleinsäuremolekül gemäß Anspruch 20, einen Antagonisten oder Agonisten identifiziert gemäß Anspruch 24, die Zusammensetzung nach Anspruch 27, die Aminosäuresequenz gemäß Anspruch 9.

26. Kit nach Anspruch 25 enthaltend Öl, Lipide oder Fettsäuren oder eine Fraktion davon gemäß Anspruch 21 oder Öl-, Lipid- oder Fettsäurezusammensetzung gemäß Anspruch 22.

27. Zusammensetzung, enthaltend den Antikörper gemäß Anspruch 19, das Antisense-Nukleinsäurekonstrukt gemäß Anspruch 20 oder einen Antagonisten oder Agonisten identifiziert gemäß Anspruch 24.

Figur 1: Polypeptidvergleich der Codierregionen von Pp_des 6 (obere Reihe) mit der EST-Sequenz von PT001078032R (untere Reihe)

```
398 WKPLVWMAVTELMSGMLLGFVFVLSHNGMEVYNSSKEFVSAQI-------------VSTR 444
    W+     + +   +    + L   +F LSHN     + S+     +A +               V T
430 WRVFGNIMLMGVAESLALAVLFSLSHN----FESADRDPTAPLKKTGEPVDWFKTQVETS 263
445 DIKGNIFNDWFTGGLNRQIEHHLFPTMPRHNLNKIAPRVEVFCKKHGLVY 494
       G    +   FTGGLN Q+EHHLFP M        IAP+V    C KHG+ Y
262 CTYGGFLSGCFTGGLNFQVEHHLFPRMSSAWYXYIAPKVREICAKHGVHY 113
```

Figur 2: Polypeptidvergleich von Codierregionen der Ma_des12 (obere Sequenz) mit PT001070010R

```
105 GVWVLAHECGHQSFSTSKTLNN 126
    G WVLAHECGH +FS +++L +
533 GFWVLAHECGHGAFSKNRSLQD 598
```

Figur 2a: Polypeptidvergleich von Codierregionen der Ma_des12 (obere Sequenz) mit PT001072031R

```
117 SFSTSKTLNNTVGWILHSMLLVPYHSWRISHSKHH 151
    ++S S+T N+ VG+I+H  LLVPY +W+ +H+KHH
465 AYSDSQTFNDVVGFIVHQALLVPYFAWQYTHAKHH 569
```

Figur 3: Polypeptidvergleich von Codierregionen eines PCR Produktes aus Primerpaar F6a und R4a2 codierend für ein Desaturase Fragment (obere Reihe) aus Phaodactylum mit der Sequenz T36617 aus Streptomyces coelicolor (untere Reihe)

```
1   WWKNKHNGHHAVPNLHCSSAVAQDGDPDIDTMPLLAWSVQQAQSYRELQADGKDSGLVKF 60
    WW++KH  HHA PN         +D DPDI    LL WS  QA++           +GL +
114 WWQDKHTRHHANPN-------TEDLDPDIGP-DLLVWSPDQARAA---------TGLPRL 156
 61  MIRNQSYFYFPILLLARLSWLNESFKCAFGLGAASENAALELKAKGLQYPLLEKAGILLH 120
     + R Q++ +FP+L L       E F      G A  N  L+ +A       L+ A +L H
157 LGRWQAFLFFPLLTL-------EGFNLHVASGRAMANRRLKRRA-------LDGALLLAH 202
121 YAWMLTVSSGFGRXXXXXXXXXXXXXXXCGFLLAIVFGLGHNGMATYNADARPDFWKLQ 180
     A  LT    F                  G  L   F   H GM    AD RPDF + Q
203 CAVYLTAL--FWVLPPGMAIAFLAVHQCLFGVYLGSAFAPNHKGMPILTADDRPDFLRRQ 260
181 VTTTRNVTGGHGFPQAFVDWFCGGLQYQVDHHLFPS 216
    V T+RNV GG      F D   GGL +Q++HHLFPS
261 VLTSRNVNGG-----LFTDLALGGLNHQIEHHLFPS 291
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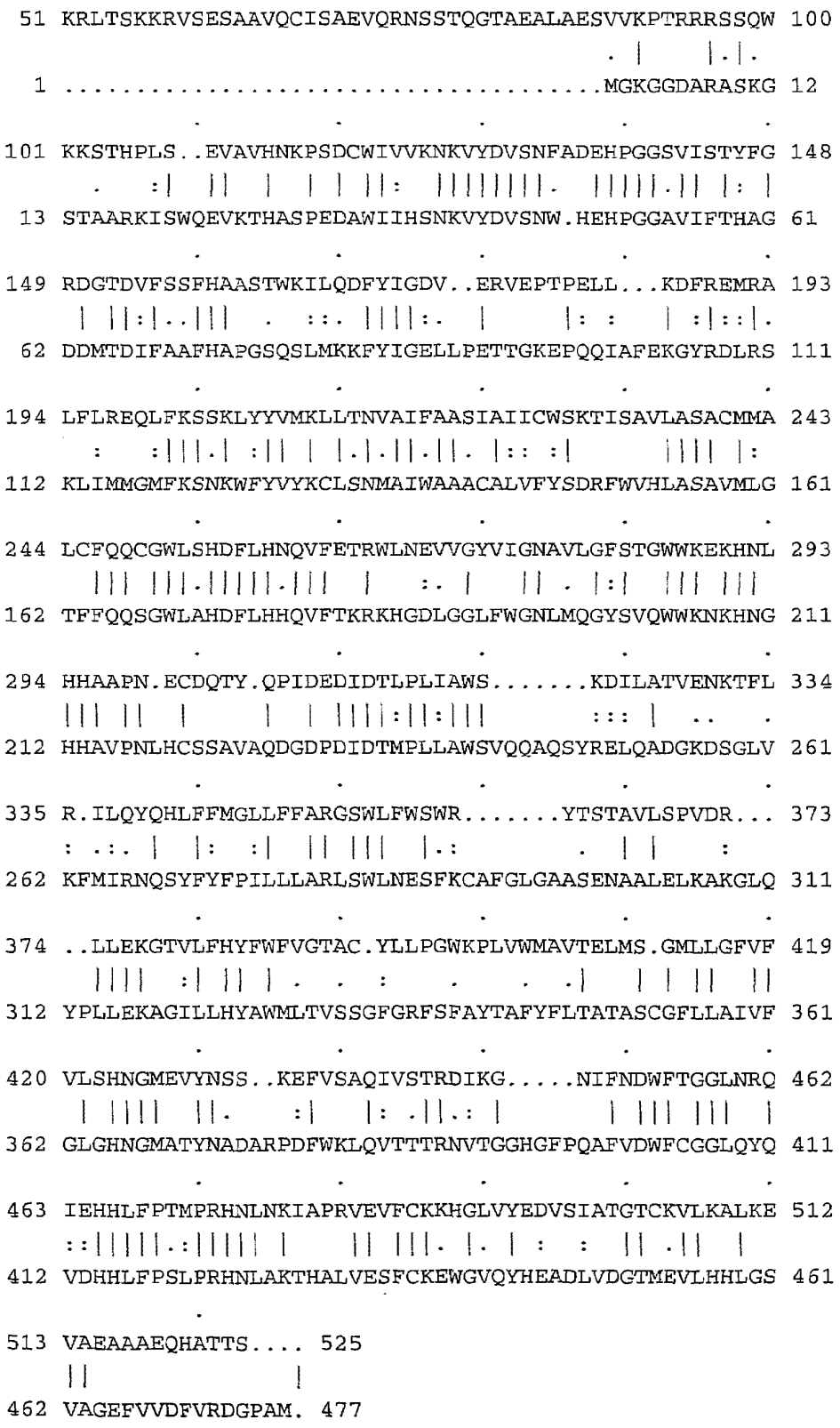
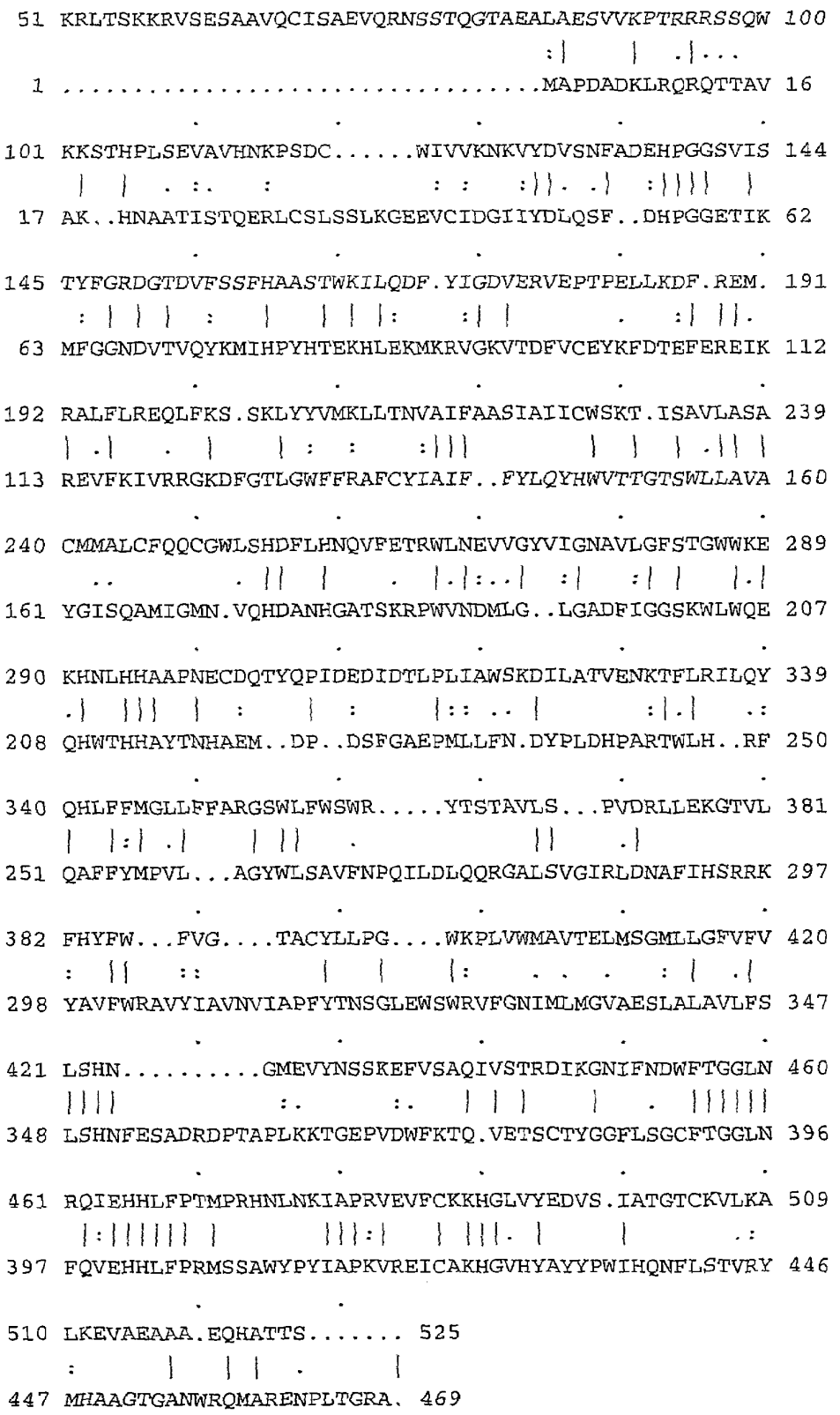
Figur 4: Polypeptidvergleich von Codierregionen aus Pp_des6 (obere Reihe) verglichen mit Pt_des6 (untere Reihe)



Figur 5: Polypeptidvergleich von Codierregionen aus Pp_des6 (obere Reihe) verglichen mit Pt_des5 (untere Reihe)



Figur 6: Polypeptidvergleich von Codierregionen der Δ-12-Desaturase aus Mortierella alpina (Ma_des12) obere Reihe mit der homologen Sequenz aus Phaeodactylum tricornutum (Pt_des12) in der unteren Reihe

```
 40 KEIRECIPAHCFERSGLRGLCHVAIDLTWASLL..FLAATQIDKFE..NP 85
    |::|  ||  |||    : | :..:  |  .:|   . |  :   :  ||
107 KDLRAVIPKDCFEPDTAKSLGYLSVS.TMGTILCSVVGANLLSVLDPSNP 155
           .        .        .        .        .
 86 LIRYLAWPVYWIMQGIVCTGVWVLAHECGHQSFSTSKTLNNTVGWILHSM 135
    |  :  |  |  . | |  |.||||||||| .|| .:.| . ||:|:||.
156 L.TWPLWAAYGAVTGTVAMGLWVLAHECGHGAFSKNRSLQDAVGYIIHSI 204
           .        .        .        .        .
136 LLVPYHSWRISHSKHHKATGHMTKDQVFVPKTRSQVGLPPKENAAAAVQE 185
    :|||| ||. ||. ||. | ||   :  ||    . |    | . |  |
205 MLVPYFSWQRSHAVHHQYTNHMELGETHVPDRADKEG....EKSLALRQF 250
           .        .        .        .        .
186 EDMSVHLDEEAPIVTLFWMVIQFLFGWPAYLIMNASGQDYGRWTSHFHTY 235
       |   |.          :  : ||||||:. |.|      |.||:
251 MLDSFGKDKGMKAYGGLQSFLHLIVGWPAYLLIGATGGPDRGMTNHFYP. 299
           .        .        .        .        .
236 SPIFEP....RNFF......DIIISDLGVLAALGALIYASMQLSLLTVTK 275
    .|:  |    :  |       :  ||:|: | .||||  .    |  |
300 NPLSTPTQPKKELFPGNWKEKVYQSDIGIAAVVGALIAWTATSGLAPVMA 349
           .        .        .        .        .
276 YYIVPYLFVNFWLVLITFLQHTDPKLPHYREGAWNFQRGALCTVDRSFGK 325
     |  | : :| |||| |.|||||  .||:     || :||| |:|| : |
350 LYGGPLIVINAWLVLYTWLQHTDTDVPHFSSDNHNFVKGALHTIDRPYDK 399
           .        .        .        .        .
326 .....FLDHMFHGIVHTHVAHHLFSQMPFYHAEEATYHLKKLLGEYYVYD 370
         :| : | |  ||||||  | .| | |: ||  :|    | |.||
400 LDPWGIIDFLHHKIGTTHVAHHFDSTIPHYKAQIATDAIKAKFPEVYLYD 449
           .        .
371 PSPIVVAVWRSFRECRFVEDQGDVVFFK 398
    |.||  |.||  : |  || .||   .|
450 PTPIPQAMWRVAKGCTAVEQRGDAWVWK 477
```

Figur 7: Polypeptidvergleich von Codierregionen der Δ-12-Desaturase aus Mortierella alpina (Ma_des12) obere Reihe mit der homologen Sequenz aus Phaeodactylum tricornutum Klon PT001072031R (Pt_des12.2) in der unteren Reihe.

```
  22 NSAKPAFERNYQLPEFTIKEIRECIPAHCFERSGLRGLCHVAIDLTWASL 71
  33 SSYNPLAKDSPELP..TKGQIKAVIPKECFQRSAFWSTFYLMRDLAMAAA 80

  72 LFLAATQIDKFENP......LIRYLAWPVYWIMQGIVCTGVWVLAHECGH 115
  81 FCYGTSQVLSTDLPQDATLILPWALGWGVYAFWMGTILTGPWVVAHECGH 130

 116 QSFSTSKTLNNTVGWILHSMLLVPYHSWRISHSKHHKATGHMTKDQVFVP 165
 131 GAYSDSQTFNDVVGFIVHQALLVPYFAWQYTHAKHHRRTNHLVDGESHVP 180

 166 KTRSQVGLPP..KENAAAAVQEEDMSVHLDEEAPIVTLFWMVIQFLFGWP 213
 181 STAKDNGLGPHNERNSFYAAWHEAMG....DGAFAVFQVWS..HLFVGWP 224

 214 AYLI.MNASGQ..DYGRW.......TSHFHTYSPIFEPRNFFDIIISDLG 253
 225 LYLAGLASTGKLAHEGWWLEERNAIADHFRPSSPMFPAKIRAKIALSSAT 274

 254 VLAALGALIYASMQLSLLTVTKYYIVPYLFVNFWLVLITFLQHTDPKLPH 303
 275 ELAVLAGLLYVGTQVGHLPVLLWYWGPYTFVNAWLVLYTWLQHTDPSIPH 324

 304 YREGAWNFQRGALCTVDRSFGKFLDHMFHGIVHTHVAHHLFSQMPFYHAE 353
 325 YGEGEWTWVKGALSTIDRDYGIF.DFFHHTIGSTHVVHHLFHEMPWYNAG 373

 354 EATYHLKKLLGE..YYVYDPSPIVVAVWRSFRECRFVEDQGDVVFFK 398
 374 IATQKVKEFLEPQGLYNYDPTPWYKAMWRIARTCHYVESNEGVQYFK 420
```

SEQUENZPROTOKOLL

<110> BASF Plant Science GmbH

<120> Verfahren zur Herstellung mehrfach ungesättigter Fettsäuren, neue Biosynthesegene sowie neue pflanzliche Expressionskonstrukte

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1

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Ala Pro Asp Ala Asp Lys Leu Arg Gln Arg Gln Thr Thr Ala Val Ala
5 10 15

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Lys His Asn Ala Ala Thr Ile Ser Thr Gln Glu Arg Leu Cys Ser Leu
20 25 30

tct tcg ctc aaa ggc gaa gaa gtc tgc atc gac gga atc atc tat gac 261
Ser Ser Leu Lys Gly Glu Glu Val Cys Ile Asp Gly Ile Ile Tyr Asp
35 40 45

ctc caa tca ttc gat cat ccc ggg ggt gaa acg atc aaa atg ttt ggt 309
Leu Gln Ser Phe Asp His Pro Gly Gly Glu Thr Ile Lys Met Phe Gly
50 55 60 65

ggc aac gat gtc act gta cag tac aag atg att cac ccg tac cat acc 357
Gly Asn Asp Val Thr Val Gln Tyr Lys Met Ile His Pro Tyr His Thr
70 75 80

gag aag cat ttg gaa aag atg aag cgt gtc ggc aag gtg acg gat ttc 405
Glu Lys His Leu Glu Lys Met Lys Arg Val Gly Lys Val Thr Asp Phe
85 90 95

gtc tgc gag tac aag ttc gat acc gaa ttt gaa cgc gaa atc aaa cga 453

```
Val Cys Glu Tyr Lys Phe Asp Thr Glu Phe Glu Arg Glu Ile Lys Arg
        100                 105                 110

gaa gtc ttc aag att gtg cga cga ggc aag gat ttc ggt act ttg gga   501
Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu Gly
    115                 120                 125

tgg ttc ttc cgt gcg ttt tgc tac att gcc att ttc ttc tac ctg cag   549
Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu Gln
130                 135                 140                 145

tac cat tgg gtc acc acg gga acc tct tgg ctg ctg gcc gtg gcc tac   597
Tyr His Trp Val Thr Thr Gly Thr Ser Trp Leu Leu Ala Val Ala Tyr
                150                 155                 160

gga atc tcc caa gcg atg att ggc atg aat gtc cag cac gat gcc aac   645
Gly Ile Ser Gln Ala Met Ile Gly Met Asn Val Gln His Asp Ala Asn
            165                 170                 175

cac ggg gcc acc tcc aag cgt ccc tgg gtc aac gac atg cta ggc ctc   693
His Gly Ala Thr Ser Lys Arg Pro Trp Val Asn Asp Met Leu Gly Leu
        180                 185                 190

ggt gcg gat ttt att ggt ggt tcc aag tgg ctc tgg cag gaa caa cac   741
Gly Ala Asp Phe Ile Gly Gly Ser Lys Trp Leu Trp Gln Glu Gln His
    195                 200                 205

tgg acc cac cac gct tac acc aat cac gcc gag atg gat ccc gat agc   789
Trp Thr His His Ala Tyr Thr Asn His Ala Glu Met Asp Pro Asp Ser
210                 215                 220                 225

ttt ggt gcc gaa cca atg ctc cta ttc aac gac tat ccc ttg gat cat   837
Phe Gly Ala Glu Pro Met Leu Leu Phe Asn Asp Tyr Pro Leu Asp His
                230                 235                 240

ccc gct cgt acc tgg cta cat cgc ttt caa gca ttc ttt tac atg ccc   885
Pro Ala Arg Thr Trp Leu His Arg Phe Gln Ala Phe Phe Tyr Met Pro
            245                 250                 255

gtc ttg gct gga tac tgg ttg tcc gct gtc ttc aat cca caa att ctt   933
Val Leu Ala Gly Tyr Trp Leu Ser Ala Val Phe Asn Pro Gln Ile Leu
        260                 265                 270

gac ctc cag caa cgc ggc gca ctt tcc gtc ggt atc cgt ctc gac aac   981
Asp Leu Gln Gln Arg Gly Ala Leu Ser Val Gly Ile Arg Leu Asp Asn
    275                 280                 285

gct ttc att cac tcg cga cgc aag tat gcg gtt ttc tgg cgg gct gtg   1029
Ala Phe Ile His Ser Arg Arg Lys Tyr Ala Val Phe Trp Arg Ala Val
290                 295                 300                 305

tac att gcg gtg aac gtg att gct ccg ttt tac aca aac tcc ggc ctc   1077
Tyr Ile Ala Val Asn Val Ile Ala Pro Phe Tyr Thr Asn Ser Gly Leu
                310                 315                 320

gaa tgg tcc tgg cgt gtc ttt gga aac atc atg ctc atg ggt gtg gcg   1125
Glu Trp Ser Trp Arg Val Phe Gly Asn Ile Met Leu Met Gly Val Ala
            325                 330                 335
```

```
gaa tcg ctc gcg ctg gcg gtc ctg ttt tcg ttg tcg cac aat ttc gaa   1173
Glu Ser Leu Ala Leu Ala Val Leu Phe Ser Leu Ser His Asn Phe Glu
        340                 345                 350

tcc gcg gat cgc gat ccg acc gcc cca ctg aaa aag acg gga gaa cca   1221
Ser Ala Asp Arg Asp Pro Thr Ala Pro Leu Lys Lys Thr Gly Glu Pro
    355                 360                 365

gtc gac tgg ttc aag aca cag gtc gaa act tcc tgc act tac ggt gga   1269
Val Asp Trp Phe Lys Thr Gln Val Glu Thr Ser Cys Thr Tyr Gly Gly
370                 375                 380                 385

ttc ctt tcc ggt tgc ttc acg gga ggt ctc aac ttt cag gtt gaa cac   1317
Phe Leu Ser Gly Cys Phe Thr Gly Gly Leu Asn Phe Gln Val Glu His
                390                 395                 400

cac ttg ttc cca cgc atg agc agc gct tgg tat ccc tac att gcc ccc   1365
His Leu Phe Pro Arg Met Ser Ser Ala Trp Tyr Pro Tyr Ile Ala Pro
            405                 410                 415

aag gtc cgc gaa att tgc gcc aaa cac ggc gtc cac tac gcc tac tac   1413
Lys Val Arg Glu Ile Cys Ala Lys His Gly Val His Tyr Ala Tyr Tyr
        420                 425                 430

ccg tgg atc cac caa aac ttt ctc tcc acc gtc cgc tac atg cac gcg   1461
Pro Trp Ile His Gln Asn Phe Leu Ser Thr Val Arg Tyr Met His Ala
    435                 440                 445

gcc ggg acc ggt gcc aac tgg cgc cag atg gcc aga gaa aat ccc ttg   1509
Ala Gly Thr Gly Ala Asn Trp Arg Gln Met Ala Arg Glu Asn Pro Leu
450                 455                 460                 465

acc gga cgg gcg taa aagtacacga cacgaccaaa ggtggcgtat ggtgatctct   1564
Thr Gly Arg Ala
                470

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            20                  25                  30

Leu Ser Ser Leu Lys Gly Glu Glu Val Cys Ile Asp Gly Ile Ile Tyr
        35                  40                  45

Asp Leu Gln Ser Phe Asp His Pro Gly Gly Glu Thr Ile Lys Met Phe
```

50 55 60

Gly Gly Asn Asp Val Thr Val Gln Tyr Lys Met Ile His Pro Tyr His
65 70 75 80

Thr Glu Lys His Leu Glu Lys Met Lys Arg Val Gly Lys Val Thr Asp
85 90 95

Phe Val Cys Glu Tyr Lys Phe Asp Thr Glu Phe Glu Arg Glu Ile Lys
100 105 110

Arg Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu
115 120 125

Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu
130 135 140

Gln Tyr His Trp Val Thr Thr Gly Thr Ser Trp Leu Leu Ala Val Ala
145 150 155 160

Tyr Gly Ile Ser Gln Ala Met Ile Gly Met Asn Val Gln His Asp Ala
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Asn His Gly Ala Thr Ser Lys Arg Pro Trp Val Asn Asp Met Leu Gly
180 185 190

Leu Gly Ala Asp Phe Ile Gly Gly Ser Lys Trp Leu Trp Gln Glu Gln
195 200 205

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Ser Phe Gly Ala Glu Pro Met Leu Leu Phe Asn Asp Tyr Pro Leu Asp
225 230 235 240

His Pro Ala Arg Thr Trp Leu His Arg Phe Gln Ala Phe Phe Tyr Met
245 250 255

Pro Val Leu Ala Gly Tyr Trp Leu Ser Ala Val Phe Asn Pro Gln Ile
260 265 270

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275 280 285

Asn Ala Phe Ile His Ser Arg Arg Lys Tyr Ala Val Phe Trp Arg Ala
290 295 300

Val Tyr Ile Ala Val Asn Val Ile Ala Pro Phe Tyr Thr Asn Ser Gly
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Leu Glu Trp Ser Trp Arg Val Phe Gly Asn Ile Met Leu Met Gly Val
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Ala Glu Ser Leu Ala Leu Ala Val Leu Phe Ser Leu Ser His Asn Phe
340 345 350

Glu Ser Ala Asp Arg Asp Pro Thr Ala Pro Leu Lys Lys Thr Gly Glu
355 360 365

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Pro Val Asp Trp Phe Lys Thr Gln Val Glu Thr Ser Cys Thr Tyr Gly
    370                 375                 380

Gly Phe Leu Ser Gly Cys Phe Thr Gly Gly Leu Asn Phe Gln Val Glu
385                 390                 395                 400

His His Leu Phe Pro Arg Met Ser Ser Ala Trp Tyr Pro Tyr Ile Ala
                405                 410                 415

Pro Lys Val Arg Glu Ile Cys Ala Lys His Gly Val His Tyr Ala Tyr
            420                 425                 430

Tyr Pro Trp Ile His Gln Asn Phe Leu Ser Thr Val Arg Tyr Met His
        435                 440                 445

Ala Ala Gly Thr Gly Ala Asn Trp Arg Gln Met Ala Arg Glu Asn Pro
    450                 455                 460

Leu Thr Gly Arg Ala
465
```

<210> 3
<211> 1434
<212> DNA
<213> Phaeodactylum tricornutum

<220>
<221> CDS
<222> (1)..(1434)

<400> 3

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atg ggc aaa gga ggg gac gct cgg gcc tcg aag ggc tca acg gcg gct   48
Met Gly Lys Gly Gly Asp Ala Arg Ala Ser Lys Gly Ser Thr Ala Ala
  1               5                  10                  15

cgc aag atc agt tgg cag gaa gtc aag acc cac gcg tct ccg gag gac   96
Arg Lys Ile Ser Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp
            20                  25                  30

gcc tgg atc att cac tcc aat aag gtc tac gac gtg tcc aac tgg cac  144
Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His
        35                  40                  45

gaa cat ccc gga ggc gcc gtc att ttc acg cac gcc ggt gac gac atg  192
Glu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
    50                  55                  60

acg gac att ttc gct gcc ttt cac gca ccc gga tcg cag tcg ctc atg  240
Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met
65                  70                  75                  80

aag aag ttc tac att ggc gaa ttg ctc ccg gaa acc acc ggc aag gag  288
Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu
                85                  90                  95

ccg cag caa atc gcc ttt gaa aag ggc tac cgc gat ctg cgc tcc aaa  336
```

```
Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys
            100                 105                 110

ctc atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac   384
Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
        115                 120                 125

aag tgc ctc agc aac atg gcc att tgg gcc gcc gcc tgt gct ctc gtc   432
Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
    130                 135                 140

ttt tac tcg gac cgc ttc tgg gta cac ctg gcc agc gcc gtc atg ctg   480
Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
145                 150                 155                 160

gga aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac   528
Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175

cac cag gtc ttc acc aag cgc aag cac ggg gat ctc gga gga ctc ttt   576
His Gln Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
            180                 185                 190

tgg ggg aac ctc atg cag ggt tac tcc gta cag tgg tgg aaa aac aag   624
Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
        195                 200                 205

cac aac gga cac cac gcc gtc ccc aac ctc cac tgc tcc tcc gca gtc   672
His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
    210                 215                 220

gcg caa gat ggg gac ccg gac atc gat acc atg ccc ctt ctc gcc tgg   720
Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
225                 230                 235                 240

tcc gtc cag caa gcc cag tct tac cgg gaa ctc caa gcc gac gga aag   768
Ser Val Gln Gln Ala Gln Ser Tyr Arg Glu Leu Gln Ala Asp Gly Lys
                245                 250                 255

gat tcg ggt ttg gtc aag ttc atg atc cgt aac caa tcc tac ttt tac   816
Asp Ser Gly Leu Val Lys Phe Met Ile Arg Asn Gln Ser Tyr Phe Tyr
            260                 265                 270

ttt ccc atc ttg ttg ctc gcc cgc ctg tcg tgg ttg aac gag tcc ttc   864
Phe Pro Ile Leu Leu Leu Ala Arg Leu Ser Trp Leu Asn Glu Ser Phe
        275                 280                 285

aag tgc gcc ttt ggg ctt gga gct gcg tcg gag aac gct gct ctc gaa   912
Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Glu Asn Ala Ala Leu Glu
    290                 295                 300

ctc aag gcc aag ggt ctt cag tac ccc ctt ttg gaa aag gct ggc atc   960
Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Glu Lys Ala Gly Ile
305                 310                 315                 320

ctg ctg cac tac gct tgg atg ctt aca gtt tcg tcc ggc ttt gga cgc  1008
Leu Leu His Tyr Ala Trp Met Leu Thr Val Ser Ser Gly Phe Gly Arg
                325                 330                 335
```

```
ttc tcg ttc gcg tac acc gca ttt tac ttt cta acc gcg acc gcg tcc   1056
Phe Ser Phe Ala Tyr Thr Ala Phe Tyr Phe Leu Thr Ala Thr Ala Ser
            340                 345                 350

tgt gga ttc ttg ctc gcc att gtc ttt ggc ctc ggc cac aac ggc atg   1104
Cys Gly Phe Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met
        355                 360                 365

gcc acc tac aat gcc gac gcc cgt ccg gac ttc tgg aag ctc caa gtc   1152
Ala Thr Tyr Asn Ala Asp Ala Arg Pro Asp Phe Trp Lys Leu Gln Val
    370                 375                 380

acc acg act cgc aac gtc acg ggc gga cac ggt ttc ccc caa gcc ttt   1200
Thr Thr Thr Arg Asn Val Thr Gly Gly His Gly Phe Pro Gln Ala Phe
385                 390                 395                 400

gtc gac tgg ttc tgt ggt ggc ctc cag tac caa gtc gac cac cac tta   1248
Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu
                405                 410                 415

ttc ccc agc ctg ccc cga cac aat ctg gcc aag aca cac gca ctg gtc   1296
Phe Pro Ser Leu Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val
            420                 425                 430

gaa tcg ttc tgc aag gag tgg ggt gtc cag tac cac gaa gcc gac ctt   1344
Glu Ser Phe Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu
        435                 440                 445

gtg gac ggg acc atg gaa gtc ttg cac cat ttg ggc agc gtg gcc ggc   1392
Val Asp Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly
    450                 455                 460

gaa ttc gtc gtg gat ttt gta cgc gat gga ccc gcc atg taa           1434
Glu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
465                 470                 475
```

<210> 4
<211> 477
<212> PRT
<213> Phaeodactylum tricornutum

<400> 4

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Met Gly Lys Gly Gly Asp Ala Arg Ala Ser Lys Gly Ser Thr Ala Ala
  1               5                  10                  15

Arg Lys Ile Ser Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp
            20                  25                  30

Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His
        35                  40                  45

Glu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
    50                  55                  60

Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met
65                  70                  75                  80
```

Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu
85 90 95

Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys
100 105 110

Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
115 120 125

Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
130 135 140

Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
145 150 155 160

Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His
165 170 175

His Gln Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
180 185 190

Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
195 200 205

His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
210 215 220

Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
225 230 235 240

Ser Val Gln Gln Ala Gln Ser Tyr Arg Glu Leu Gln Ala Asp Gly Lys
245 250 255

Asp Ser Gly Leu Val Lys Phe Met Ile Arg Asn Gln Ser Tyr Phe Tyr
260 265 270

Phe Pro Ile Leu Leu Leu Ala Arg Leu Ser Trp Leu Asn Glu Ser Phe
275 280 285

Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Glu Asn Ala Ala Leu Glu
290 295 300

Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Glu Lys Ala Gly Ile
305 310 315 320

Leu Leu His Tyr Ala Trp Met Leu Thr Val Ser Ser Gly Phe Gly Arg
325 330 335

Phe Ser Phe Ala Tyr Thr Ala Phe Tyr Phe Leu Thr Ala Thr Ala Ser
340 345 350

Cys Gly Phe Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met
355 360 365

Ala Thr Tyr Asn Ala Asp Ala Arg Pro Asp Phe Trp Lys Leu Gln Val
370 375 380

```
Thr Thr Thr Arg Asn Val Thr Gly Gly His Gly Phe Pro Gln Ala Phe
385                 390                 395                 400

Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu
                405                 410                 415

Phe Pro Ser Leu Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val
            420                 425                 430

Glu Ser Phe Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu
        435                 440                 445

Val Asp Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly
    450                 455                 460

Glu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
465                 470                 475


<210> 5
<211> 1651
<212> DNA
<213> Phaeodactylum tricornutum

<220>
<221> CDS
<222> (67)..(1554)

<400> 5
gaagaaggaa catataaaag taagccatct cctcggcacc atctaaagac ctaatatcta 60

ctcgtc atg gtt cgc ttt tca aca gcc gct cta ctt tct ctg tcg aca    108
       Met Val Arg Phe Ser Thr Ala Ala Leu Leu Ser Leu Ser Thr
         1               5                  10

ttg aca act tca tgt att ggt gcc ttc cag ctg tct tcg cca gca caa   156
Leu Thr Thr Ser Cys Ile Gly Ala Phe Gln Leu Ser Ser Pro Ala Gln
 15                  20                  25                  30

ctt ccg aca agt agg ctt cgt cgg cat acg aac acg gcg ccg ctt tcg   204
Leu Pro Thr Ser Arg Leu Arg Arg His Thr Asn Thr Ala Pro Leu Ser
             35                  40                  45

gcc gtg gcc gtc gac tcc ggt tct tcc gat ccg gcc ttg gta ggc aac   252
Ala Val Ala Val Asp Ser Gly Ser Ser Asp Pro Ala Leu Val Gly Asn
         50                  55                  60

ctc ccc ctt ccc aac aac aat gat aat gag gac aag aac cgt aga atg   300
Leu Pro Leu Pro Asn Asn Asn Asp Asn Glu Asp Lys Asn Arg Arg Met
     65                  70                  75

cca atg atg gac ttg aaa ggt att gct ctg tct ggt ctc aaa ggg caa   348
Pro Met Met Asp Leu Lys Gly Ile Ala Leu Ser Gly Leu Lys Gly Gln
 80                  85                  90

gct ctt tcc gtc cga gcg gaa gat ttt cct cag gcg aaa gac ttg cgt   396
Ala Leu Ser Val Arg Ala Glu Asp Phe Pro Gln Ala Lys Asp Leu Arg
 95                  100                 105                 110
```

```
gcc gtc att ccg aaa gat tgc ttc gaa ccc gac acg gcc aaa tcg ttg   444
Ala Val Ile Pro Lys Asp Cys Phe Glu Pro Asp Thr Ala Lys Ser Leu
                115                 120                 125

gga tat ctt tcc gtt tca act atg ggg aca att ctc tgc tcc gtc gtc   492
Gly Tyr Leu Ser Val Ser Thr Met Gly Thr Ile Leu Cys Ser Val Val
            130                 135                 140

ggc gcg aac ctc ctt agt gtg ctc gat ccc tcc aat cca tta acc tgg   540
Gly Ala Asn Leu Leu Ser Val Leu Asp Pro Ser Asn Pro Leu Thr Trp
        145                 150                 155

cct ctc tgg gcg gcc tac ggt gcc gtc acg ggg acg gtc gcc atg ggg   588
Pro Leu Trp Ala Ala Tyr Gly Ala Val Thr Gly Thr Val Ala Met Gly
    160                 165                 170

ctt tgg gtg ctg gcc cac gaa tgc gga cac ggc gcc ttt tcc aaa aac   636
Leu Trp Val Leu Ala His Glu Cys Gly His Gly Ala Phe Ser Lys Asn
175                 180                 185                 190

cga tcc ctc cag gat gcc gtg ggg tac att atc cat tcc atc atg ctg   684
Arg Ser Leu Gln Asp Ala Val Gly Tyr Ile Ile His Ser Ile Met Leu
                195                 200                 205

gtg cca tac ttt agt tgg cag cga tcg cat gcc gtg cat cac cag tat   732
Val Pro Tyr Phe Ser Trp Gln Arg Ser His Ala Val His His Gln Tyr
            210                 215                 220

acc aat cat atg gaa ctg ggg gaa aca cac gtt cct gat cga gcc gat   780
Thr Asn His Met Glu Leu Gly Glu Thr His Val Pro Asp Arg Ala Asp
        225                 230                 235

aag gag ggc gag aag agc ctg gcg ctc cgc cag ttc atg ttg gat tcc   828
Lys Glu Gly Glu Lys Ser Leu Ala Leu Arg Gln Phe Met Leu Asp Ser
    240                 245                 250

ttt ggt aaa gac aag ggc atg aaa gca tac gga ggc ctc cag tcg ttt   876
Phe Gly Lys Asp Lys Gly Met Lys Ala Tyr Gly Gly Leu Gln Ser Phe
255                 260                 265                 270

ttg cat ctc atc gtg gga tgg cca gcc tac ctc ctg atc ggt gcg acc   924
Leu His Leu Ile Val Gly Trp Pro Ala Tyr Leu Leu Ile Gly Ala Thr
                275                 280                 285

ggt gga ccc gac cgt ggt atg acc aac cat ttt tat ccc aac cct ttg   972
Gly Gly Pro Asp Arg Gly Met Thr Asn His Phe Tyr Pro Asn Pro Leu
            290                 295                 300

tcg acg cca aca cag ccc aag aaa gaa ctt ttc cct ggg aac tgg aaa  1020
Ser Thr Pro Thr Gln Pro Lys Lys Glu Leu Phe Pro Gly Asn Trp Lys
        305                 310                 315

gaa aag gtc tac cag tca gat att gga atc gcc gcc gtt gtc ggc gcc  1068
Glu Lys Val Tyr Gln Ser Asp Ile Gly Ile Ala Ala Val Val Gly Ala
    320                 325                 330

ctc att gct tgg acc gcc act tcg ggt cta gcc ccc gtc atg gcc ttg  1116
```

```
Leu Ile Ala Trp Thr Ala Thr Ser Gly Leu Ala Pro Val Met Ala Leu
335                 340                 345                 350

tac ggt ggt ccc ttg atc gtc att aat gcc tgg ctg gta ctg tac acg   1164
Tyr Gly Gly Pro Leu Ile Val Ile Asn Ala Trp Leu Val Leu Tyr Thr
                355                 360                 365

tgg ttg caa cat aca gat acc gat gtt ccg cac ttt tcc tcc gac aac   1212
Trp Leu Gln His Thr Asp Thr Asp Val Pro His Phe Ser Ser Asp Asn
            370                 375                 380

cac aac ttt gtc aag ggc gca ctg cat acg atc gat cgt ccc tac gac   1260
His Asn Phe Val Lys Gly Ala Leu His Thr Ile Asp Arg Pro Tyr Asp
        385                 390                 395

aaa ctt gat ccc tgg gga atc ata gac ttt ctg cac cac aag att gga   1308
Lys Leu Asp Pro Trp Gly Ile Ile Asp Phe Leu His His Lys Ile Gly
    400                 405                 410

aca acg cat gtg gca cac cat ttt gac agt act atc ccc cac tat aag   1356
Thr Thr His Val Ala His His Phe Asp Ser Thr Ile Pro His Tyr Lys
415                 420                 425                 430

gct cag att gct acc gat gcc atc aaa gcc aag ttt cca gaa gtg tac   1404
Ala Gln Ile Ala Thr Asp Ala Ile Lys Ala Lys Phe Pro Glu Val Tyr
                435                 440                 445

ctc tat gac ccg aca cca att cca caa gcc atg tgg cgc gtc gcc aag   1452
Leu Tyr Asp Pro Thr Pro Ile Pro Gln Ala Met Trp Arg Val Ala Lys
            450                 455                 460

gga tgt act gca gta gag caa cgc ggt gac gcc tgg gtg tgg aaa aac   1500
Gly Cys Thr Ala Val Glu Gln Arg Gly Asp Ala Trp Val Trp Lys Asn
        465                 470                 475

gaa gga ata gaa gat ttg gtg gaa cat cgt caa agc aaa tta tcg agc   1548
Glu Gly Ile Glu Asp Leu Val Glu His Arg Gln Ser Lys Leu Ser Ser
    480                 485                 490

gaa taa agcaacatat cgctttatgg aagaacaaac gtccattgtg taaaaccctg   1604
Glu
495

ataatttcaa tattgtgttt tgttttaaaa aaaaaaaaaa aaaaaaa              1651


<210> 6
<211> 495
<212> PRT
<213> Phaeodactylum tricornutum

<400> 6
Met Val Arg Phe Ser Thr Ala Ala Leu Leu Ser Leu Ser Thr Leu Thr
  1               5                  10                  15

Thr Ser Cys Ile Gly Ala Phe Gln Leu Ser Ser Pro Ala Gln Leu Pro
             20                  25                  30
```

Thr Ser Arg Leu Arg Arg His Thr Asn Thr Ala Pro Leu Ser Ala Val
35 40 45

Ala Val Asp Ser Gly Ser Ser Asp Pro Ala Leu Val Gly Asn Leu Pro
50 55 60

Leu Pro Asn Asn Asn Asp Asn Glu Asp Lys Asn Arg Arg Met Pro Met
65 70 75 80

Met Asp Leu Lys Gly Ile Ala Leu Ser Gly Leu Lys Gly Gln Ala Leu
85 90 95

Ser Val Arg Ala Glu Asp Phe Pro Gln Ala Lys Asp Leu Arg Ala Val
100 105 110

Ile Pro Lys Asp Cys Phe Glu Pro Asp Thr Ala Lys Ser Leu Gly Tyr
115 120 125

Leu Ser Val Ser Thr Met Gly Thr Ile Leu Cys Ser Val Val Gly Ala
130 135 140

Asn Leu Leu Ser Val Leu Asp Pro Ser Asn Pro Leu Thr Trp Pro Leu
145 150 155 160

Trp Ala Ala Tyr Gly Ala Val Thr Gly Thr Val Ala Met Gly Leu Trp
165 170 175

Val Leu Ala His Glu Cys Gly His Gly Ala Phe Ser Lys Asn Arg Ser
180 185 190

Leu Gln Asp Ala Val Gly Tyr Ile Ile His Ser Ile Met Leu Val Pro
195 200 205

Tyr Phe Ser Trp Gln Arg Ser His Ala Val His His Gln Tyr Thr Asn
210 215 220

His Met Glu Leu Gly Glu Thr His Val Pro Asp Arg Ala Asp Lys Glu
225 230 235 240

Gly Glu Lys Ser Leu Ala Leu Arg Gln Phe Met Leu Asp Ser Phe Gly
245 250 255

Lys Asp Lys Gly Met Lys Ala Tyr Gly Gly Leu Gln Ser Phe Leu His
260 265 270

Leu Ile Val Gly Trp Pro Ala Tyr Leu Leu Ile Gly Ala Thr Gly Gly
275 280 285

Pro Asp Arg Gly Met Thr Asn His Phe Tyr Pro Asn Pro Leu Ser Thr
290 295 300

Pro Thr Gln Pro Lys Lys Glu Leu Phe Pro Gly Asn Trp Lys Glu Lys
305 310 315 320

Val Tyr Gln Ser Asp Ile Gly Ile Ala Ala Val Val Gly Ala Leu Ile
325 330 335

Ala Trp Thr Ala Thr Ser Gly Leu Ala Pro Val Met Ala Leu Tyr Gly

```
            340                 345                 350

Gly Pro Leu Ile Val Ile Asn Ala Trp Leu Val Leu Tyr Thr Trp Leu
        355                 360                 365

Gln His Thr Asp Thr Asp Val Pro His Phe Ser Ser Asp Asn His Asn
    370                 375                 380

Phe Val Lys Gly Ala Leu His Thr Ile Asp Arg Pro Tyr Asp Lys Leu
385                 390                 395                 400

Asp Pro Trp Gly Ile Ile Asp Phe Leu His His Lys Ile Gly Thr Thr
                405                 410                 415

His Val Ala His His Phe Asp Ser Thr Ile Pro His Tyr Lys Ala Gln
            420                 425                 430

Ile Ala Thr Asp Ala Ile Lys Ala Lys Phe Pro Glu Val Tyr Leu Tyr
        435                 440                 445

Asp Pro Thr Pro Ile Pro Gln Ala Met Trp Arg Val Ala Lys Gly Cys
    450                 455                 460

Thr Ala Val Glu Gln Arg Gly Asp Ala Trp Val Trp Lys Asn Glu Gly
465                 470                 475                 480

Ile Glu Asp Leu Val Glu His Arg Gln Ser Lys Leu Ser Ser Glu
                485                 490                 495


<210> 7
<211> 1578
<212> DNA
<213> Physcomitrella patens

<220>
<221> CDS
<222> (1)..(1578)

<400> 7
atg gta ttc gcg ggc ggt gga ctt cag cag ggc tct ctc gaa gaa aac    48
Met Val Phe Ala Gly Gly Gly Leu Gln Gln Gly Ser Leu Glu Glu Asn
  1               5                  10                  15

atc gac gtc gag cac att gcc agt atg tct ctc ttc agc gac ttc ttc    96
Ile Asp Val Glu His Ile Ala Ser Met Ser Leu Phe Ser Asp Phe Phe
            20                  25                  30

agt tat gtg tct tca act gtt ggt tcg tgg agc gta cac agt ata caa   144
Ser Tyr Val Ser Ser Thr Val Gly Ser Trp Ser Val His Ser Ile Gln
        35                  40                  45

cct ttg aag cgc ctg acg agt aag aag cgt gtt tcg gaa agc gct gcc   192
Pro Leu Lys Arg Leu Thr Ser Lys Lys Arg Val Ser Glu Ser Ala Ala
    50                  55                  60

gtg caa tgt ata tca gct gaa gtt cag aga aat tcg agt acc cag gga   240
Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
```

```
65                  70                  75                  80

act gcg gag gca ctc gca gaa tca gtc gtg aag ccc acg aga cga agg   288
Thr Ala Glu Ala Leu Ala Glu Ser Val Val Lys Pro Thr Arg Arg Arg
                85                  90                  95

tca tct cag tgg aag aag tcg aca cac ccc cta tca gaa gta gca gta   336
Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
            100                 105                 110

cac aac aag cca agc gat tgc tgg att gtt gta aaa aac aag gtg tat   384
His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
        115                 120                 125

gat gtt tcc aat ttt gcg gac gag cat ccc gga gga tca gtt att agt   432
Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
    130                 135                 140

act tat ttt gga cga gac ggc aca gat gtt ttc tct agt ttt cat gca   480
Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
145                 150                 155                 160

gct tct aca tgg aaa att ctt caa gac ttt tac att ggt gac gtg gag   528
Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
                165                 170                 175

agg gtg gag ccg act cca gag ctg ctg aaa gat ttc cga gaa atg aga   576
Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
            180                 185                 190

gct ctt ttc ctg agg gag caa ctt ttc aaa agt tcg aaa ttg tac tat   624
Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
        195                 200                 205

gtt atg aag ctg ctc acg aat gtt gct att ttt gct gcg agc att gca   672
Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
    210                 215                 220

ata ata tgt tgg agc aag act att tca gcg gtt ttg gct tca gct tgt   720
Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
225                 230                 235                 240

atg atg gct ctg tgt ttc caa cag tgc gga tgg cta tcc cat gat ttt   768
Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
                245                 250                 255

ctc cac aat cag gtg ttt gag aca cgc tgg ctt aat gaa gtt gtc ggg   816
Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
            260                 265                 270

tat gtg atc ggc aac gcc gtt ctg ggg ttt agt aca ggg tgg tgg aag   864
Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
        275                 280                 285

gag aag cat aac ctt cat cat gct gct cca aat gaa tgc gat cag act   912
Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
    290                 295                 300
```

```
tac caa cca att gat gaa gat att gat act ctc ccc ctc att gcc tgg   960
Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
305                 310                 315                 320

agc aag gac ata ctg gcc aca gtt gag aat aag aca ttc ttg cga atc   1008
Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
                325                 330                 335

ctc caa tac cag cat ctg ttc ttc atg ggt ctg tta ttt ttc gcc cgt   1056
Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
            340                 345                 350

ggt agt tgg ctc ttt tgg agc tgg aga tat acc tct aca gca gtg ctc   1104
Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
        355                 360                 365

tca cct gtc gac agg ttg ttg gag aag gga act gtt ctg ttt cac tac   1152
Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
    370                 375                 380

ttt tgg ttc gtc ggg aca gcg tgc tat ctt ctc cct ggt tgg aag cca   1200
Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
385                 390                 395                 400

tta gta tgg atg gcg gtg act gag ctc atg tcc ggc atg ctg ctg ggc   1248
Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
                405                 410                 415

ttt gta ttt gta ctt agc cac aat ggg atg gag gtt tat aat tcg tct   1296
Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
            420                 425                 430

aaa gaa ttc gtg agt gca cag atc gta tcc aca cgg gat atc aaa gga   1344
Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
        435                 440                 445

aac ata ttc aac gac tgg ttc act ggt ggc ctt aac agg caa ata gag   1392
Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu
    450                 455                 460

cat cat ctt ttc cca aca atg ccc agg cat aat tta aac aaa ata gca   1440
His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Lys Ile Ala
465                 470                 475                 480

cct aga gtg gag gtg ttc tgt aag aaa cac ggt ctg gtg tac gaa gac   1488
Pro Arg Val Glu Val Phe Cys Lys Lys His Gly Leu Val Tyr Glu Asp
                485                 490                 495

gta tct att gct acc ggc act tgc aag gtt ttg aaa gca ttg aag gaa   1536
Val Ser Ile Ala Thr Gly Thr Cys Lys Val Leu Lys Ala Leu Lys Glu
            500                 505                 510

gtc gcg gag gct gcg gca gag cag cat gct acc acc agt taa           1578
Val Ala Glu Ala Ala Ala Glu Gln His Ala Thr Thr Ser
        515                 520                 525
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<210> 8

<211> 525
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<213> Physcomitrella patens

<400> 8

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Ile Asp Val Glu His Ile Ala Ser Met Ser Leu Phe Ser Asp Phe Phe
            20                  25                  30

Ser Tyr Val Ser Ser Thr Val Gly Ser Trp Ser Val His Ser Ile Gln
        35                  40                  45

Pro Leu Lys Arg Leu Thr Ser Lys Lys Arg Val Ser Glu Ser Ala Ala
    50                  55                  60

Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
65                  70                  75                  80

Thr Ala Glu Ala Leu Ala Glu Ser Val Val Lys Pro Thr Arg Arg Arg
                85                  90                  95

Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
            100                 105                 110

His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
        115                 120                 125

Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
    130                 135                 140

Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
145                 150                 155                 160

Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
                165                 170                 175

Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
            180                 185                 190

Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
        195                 200                 205

Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
    210                 215                 220

Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
225                 230                 235                 240

Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
                245                 250                 255

Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
            260                 265                 270

Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
        275                 280                 285
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Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
290 295 300

Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
305 310 315 320

Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
325 330 335

Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
340 345 350

Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
355 360 365

Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
370 375 380

Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
385 390 395 400

Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
405 410 415

Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
420 425 430

Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
435 440 445

Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu
450 455 460

His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Lys Ile Ala
465 470 475 480

Pro Arg Val Glu Val Phe Cys Lys Lys His Gly Leu Val Tyr Glu Asp
485 490 495

Val Ser Ile Ala Thr Gly Thr Cys Lys Val Leu Lys Ala Leu Lys Glu
500 505 510

Val Ala Glu Ala Ala Ala Glu Gln His Ala Thr Thr Ser
515 520 525

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cag ggc gtg aat gca ttg ctg ggt agt ttt ggg gtg gag ttg acg gat   96
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30

acg ccc act acc aaa ggc ttg ccc ctc gtt gac agt ccc aca ccc atc   144
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45

gtc ctc ggt gtt tct gta tac ttg act att gtc att gga ggg ctt ttg   192
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60

tgg ata aag gcc agg gat ctg aaa ccg cgc gcc tcg gag cca ttt ttg   240
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80

ctc caa gct ttg gtg ctt gtg cac aac ctg ttc tgt ttt gcg ctc agt   288
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95

ctg tat atg tgc gtg ggc atc gct tat cag gct att acc tgg cgg tac   336
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110

tct ctc tgg ggc aat gca tac aat cct aaa cat aaa gag atg gcg att   384
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125

ctg gta tac ttg ttc tac atg tct aag tac gtg gaa ttc atg gat acc   432
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140

gtt atc atg ata ctg aag cgc agc acc agg caa ata agc ttc ctc cac   480
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160

gtt tat cat cat tct tca att tcc ctc att tgg tgg gct att gct cat   528
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175

cac gct cct ggc ggt gaa gca tat tgg tct gcg gct ctg aac tca gga   576
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190

gtg cat gtt ctc atg tat gcg tat tac ttc ttg gct gcc tgc ctt cga   624
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205

agt agc cca aag tta aaa aat aag tac ctt ttt tgg ggc agg tac ttg   672
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220

aca caa ttc caa atg ttc cag ttt atg ctg aac tta gtg cag gct tac   720
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240
```

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tac gac atg aaa acg aat gcg cca tat cca caa tgg ctg atc aag att   768
Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255

ttg ttc tac tac atg atc tcg ttg ctg ttt ctt ttc ggc aat ttt tac   816
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
            260                 265                 270

gta caa aaa tac atc aaa ccc tct gac gga aag caa aag gga gct aaa   864
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
        275                 280                 285

act gag tga                                                      873
Thr Glu
    290
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<210> 10
<211> 290
<212> PRT
<213> Physcomitrella patens

<400> 10

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Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
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Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
            20                  25                  30

Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
        35                  40                  45

Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
    50                  55                  60

Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65                  70                  75                  80

Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95

Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110

Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125

Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140

Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160

Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175

His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
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            180                 185                 190

Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205

Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220

Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240

Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255

Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
            260                 265                 270

Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
        275                 280                 285

Thr Glu
    290


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<213> Phaeodactylum tricornutum

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<222> (92)..(1402)

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tacttactac aacgaagcaa ccaccagcag c atg ggt aag gga ggt caa cga    112
                                    Met Gly Lys Gly Gly Gln Arg
                                      1               5

gct gta gct ccc aag agt gcc acc agc tct act ggc agt gct acc ctt   160
Ala Val Ala Pro Lys Ser Ala Thr Ser Ser Thr Gly Ser Ala Thr Leu
         10                  15                  20

agc caa agc aag gaa cag gta tgg act tcg tcg tac aac cct ctg gcg   208
Ser Gln Ser Lys Glu Gln Val Trp Thr Ser Ser Tyr Asn Pro Leu Ala
     25                  30                  35

aag gat tcc ccg gag ctg cca acc aaa ggc caa atc aag gcc gtc att   256
Lys Asp Ser Pro Glu Leu Pro Thr Lys Gly Gln Ile Lys Ala Val Ile
 40                  45                  50                  55

ccg aag gaa tgt ttc caa cgc tca gcc ttt tgg tct acc ttc tac ctg   304
Pro Lys Glu Cys Phe Gln Arg Ser Ala Phe Trp Ser Thr Phe Tyr Leu
                 60                  65                  70

atg cgc gat ctc gcc atg gct gcc gcc ttt tgc tac gga acc tca cag   352
Met Arg Asp Leu Ala Met Ala Ala Ala Phe Cys Tyr Gly Thr Ser Gln
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                75                  80                  85

gtc ctc tcc acc gac ctt ccc caa gac gcc acg ctc att ctg ccc tgg   400
Val Leu Ser Thr Asp Leu Pro Gln Asp Ala Thr Leu Ile Leu Pro Trp
         90                  95                 100

gct ctc ggc tgg ggc gtc tac gcc ttt tgg atg gga acc att ctc acc   448
Ala Leu Gly Trp Gly Val Tyr Ala Phe Trp Met Gly Thr Ile Leu Thr
    105                 110                 115

ggg cct tgg gta gtt gcg cac gaa tgt gga cac ggc gct tac tcc gac   496
Gly Pro Trp Val Val Ala His Glu Cys Gly His Gly Ala Tyr Ser Asp
120                 125                 130                 135

tcc cag acg ttc aat gac gtg gtc ggc ttt atc gtc cac caa gct ttg   544
Ser Gln Thr Phe Asn Asp Val Val Gly Phe Ile Val His Gln Ala Leu
            140                 145                 150

ctc gtc ccc tac ttt gcc tgg cag tac acc cac gcg aaa cac cac cgt   592
Leu Val Pro Tyr Phe Ala Trp Gln Tyr Thr His Ala Lys His His Arg
        155                 160                 165

cga acc aac cat ctg gtg gac ggc gag tcc cac gtc cct tct acc gcc   640
Arg Thr Asn His Leu Val Asp Gly Glu Ser His Val Pro Ser Thr Ala
    170                 175                 180

aag gat aac ggc ctc ggg ccg cac aac gag cga aac tcc ttc tac gcc   688
Lys Asp Asn Gly Leu Gly Pro His Asn Glu Arg Asn Ser Phe Tyr Ala
185                 190                 195

gcg tgg cac gag gcc atg gga gac ggc gcc ttt gcc gtc ttt caa gtc   736
Ala Trp His Glu Ala Met Gly Asp Gly Ala Phe Ala Val Phe Gln Val
200                 205                 210                 215

tgg tcg cac ttg ttc gtc ggc tgg cct ctc tac ttg gcc ggt ctg gcc   784
Trp Ser His Leu Phe Val Gly Trp Pro Leu Tyr Leu Ala Gly Leu Ala
            220                 225                 230

agt acc gga aag ctt gcg cac gaa ggt tgg tgg ctg gaa gaa cgg aac   832
Ser Thr Gly Lys Leu Ala His Glu Gly Trp Trp Leu Glu Glu Arg Asn
        235                 240                 245

gcg att gcg gat cac ttt cga ccc agc tct ccc atg ttc ccc gcc aag   880
Ala Ile Ala Asp His Phe Arg Pro Ser Ser Pro Met Phe Pro Ala Lys
    250                 255                 260

atc cgt gcc aag att gcc ctt tcc agc gcg acg gaa ctc gcc gtg ctc   928
Ile Arg Ala Lys Ile Ala Leu Ser Ser Ala Thr Glu Leu Ala Val Leu
265                 270                 275

gct gga ctc ttg tat gtc ggt aca cag gtc gga cac ctt ccc gtc ctg   976
Ala Gly Leu Leu Tyr Val Gly Thr Gln Val Gly His Leu Pro Val Leu
280                 285                 290                 295

ctg tgg tac tgg gga ccg tac acc ttt gtc aac gct tgg ctt gta ctc  1024
Leu Trp Tyr Trp Gly Pro Tyr Thr Phe Val Asn Ala Trp Leu Val Leu
            300                 305                 310
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tac acg tgg ctg cag cat acg gac ccg tcc atc ccg cac tac ggt gaa   1072
Tyr Thr Trp Leu Gln His Thr Asp Pro Ser Ile Pro His Tyr Gly Glu
            315                 320                 325

ggc gag tgg acc tgg gtc aag ggc gcg ctc tct acc att gat cga gac   1120
Gly Glu Trp Thr Trp Val Lys Gly Ala Leu Ser Thr Ile Asp Arg Asp
        330                 335                 340

tac ggc atc ttc gat ttc ttt cac cac acc atc ggt tcc acg cac gtg   1168
Tyr Gly Ile Phe Asp Phe Phe His His Thr Ile Gly Ser Thr His Val
    345                 350                 355

gta cac cat ttg ttc cac gaa atg ccc tgg tac aat gcc ggc att gcc   1216
Val His His Leu Phe His Glu Met Pro Trp Tyr Asn Ala Gly Ile Ala
360                 365                 370                 375

acg caa aag gtc aag gaa ttt ttg gaa ccc cag ggc ttg tac aat tac   1264
Thr Gln Lys Val Lys Glu Phe Leu Glu Pro Gln Gly Leu Tyr Asn Tyr
                380                 385                 390

gat ccg acc ccc tgg tac aag gcc atg tgg cgc att gcc cgg acc tgt   1312
Asp Pro Thr Pro Trp Tyr Lys Ala Met Trp Arg Ile Ala Arg Thr Cys
            395                 400                 405

cac tat gtg gag tca aac gag ggt gtg cag tat ttc aag agt atg gaa   1360
His Tyr Val Glu Ser Asn Glu Gly Val Gln Tyr Phe Lys Ser Met Glu
        410                 415                 420

aac gtg ccg ctg act aag gat gtg cga aac aaa gcc gca tga           1402
Asn Val Pro Leu Thr Lys Asp Val Arg Asn Lys Ala Ala
    425                 430                 435

gaaaaagtgc caccgacgca taattttaca atcctaccaa caagaccaac attatatggt 1462

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<213> Phaeodactylum tricornutum

<400> 12

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            20                  25                  30

Ser Ser Tyr Asn Pro Leu Ala Lys Asp Ser Pro Glu Leu Pro Thr Lys
        35                  40                  45

Gly Gln Ile Lys Ala Val Ile Pro Lys Glu Cys Phe Gln Arg Ser Ala
    50                  55                  60

Phe Trp Ser Thr Phe Tyr Leu Met Arg Asp Leu Ala Met Ala Ala Ala
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65 70 75 80

Phe Cys Tyr Gly Thr Ser Gln Val Leu Ser Thr Asp Leu Pro Gln Asp
85 90 95

Ala Thr Leu Ile Leu Pro Trp Ala Leu Gly Trp Gly Val Tyr Ala Phe
100 105 110

Trp Met Gly Thr Ile Leu Thr Gly Pro Trp Val Val Ala His Glu Cys
115 120 125

Gly His Gly Ala Tyr Ser Asp Ser Gln Thr Phe Asn Asp Val Val Gly
130 135 140

Phe Ile Val His Gln Ala Leu Leu Val Pro Tyr Phe Ala Trp Gln Tyr
145 150 155 160

Thr His Ala Lys His His Arg Arg Thr Asn His Leu Val Asp Gly Glu
165 170 175

Ser His Val Pro Ser Thr Ala Lys Asp Asn Gly Leu Gly Pro His Asn
180 185 190

Glu Arg Asn Ser Phe Tyr Ala Ala Trp His Glu Ala Met Gly Asp Gly
195 200 205

Ala Phe Ala Val Phe Gln Val Trp Ser His Leu Phe Val Gly Trp Pro
210 215 220

Leu Tyr Leu Ala Gly Leu Ala Ser Thr Gly Lys Leu Ala His Glu Gly
225 230 235 240

Trp Trp Leu Glu Glu Arg Asn Ala Ile Ala Asp His Phe Arg Pro Ser
245 250 255

Ser Pro Met Phe Pro Ala Lys Ile Arg Ala Lys Ile Ala Leu Ser Ser
260 265 270

Ala Thr Glu Leu Ala Val Leu Ala Gly Leu Leu Tyr Val Gly Thr Gln
275 280 285

Val Gly His Leu Pro Val Leu Leu Trp Tyr Trp Gly Pro Tyr Thr Phe
290 295 300

Val Asn Ala Trp Leu Val Leu Tyr Thr Trp Leu Gln His Thr Asp Pro
305 310 315 320

Ser Ile Pro His Tyr Gly Glu Gly Glu Trp Thr Trp Val Lys Gly Ala
325 330 335

Leu Ser Thr Ile Asp Arg Asp Tyr Gly Ile Phe Asp Phe Phe His His
340 345 350

Thr Ile Gly Ser Thr His Val Val His His Leu Phe His Glu Met Pro
355 360 365

Trp Tyr Asn Ala Gly Ile Ala Thr Gln Lys Val Lys Glu Phe Leu Glu
370 375 380

Pro Gln Gly Leu Tyr Asn Tyr Asp Pro Thr Pro Trp Tyr Lys Ala Met
385 390 395 400

Trp Arg Ile Ala Arg Thr Cys His Tyr Val Glu Ser Asn Glu Gly Val
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Gln Tyr Phe Lys Ser Met Glu Asn Val Pro Leu Thr Lys Asp Val Arg
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<210> 13
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Promotor-Terminator-Expressionskassette in Vektor
pUC19 dar

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aagggaataa gggcgacacg gaaatgttga atactcatac tcttcctttt tcaatattat 3420

tgaagcattt atcagggtta ttgtctcatg agcggataca tatttgaatg tatttagaaa 3480

aataaacaaa taggggttcc gcgcacattt ccccgaaaag tgccacctga cgtctaagaa 3540

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<210> 14
<211> 3590
<212> DNA
<213> Unknown

<220>
<223> Sequenz stellt eine pflanzliche
Promotor-Terminator-Expressionskassette in Vektor
pUC19 dar

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accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cggcgcgccg agctcctcga 420

gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

tttatgctaa cgtttgccaa cacttagcaa tttgcaagtt gattaattga ttctaaatta 600

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agacaacaat gttaccacac acaagttttg aggtgcatgc atggatgccc tgtggaaagt 900

ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960

ggaggatgca ataatgaaga aaactacaaa tttacatgca actagttatg catgtagtct 1020

atataatgag gattttgcaa tactttcatt catacacact cactaagttt tacacgatta 1080

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gttatcactc atggttatgg cagcactgca taattctctt actgtcatgc catccgtaag 3060

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gttgagatcc agttcgatgt aacccactcg tgcacccaac tgatcttcag catcttttac 3300

tttcaccagc gtttctgggt gagcaaaaac aggaaggcaa aatgccgcaa aaaagggaat 3360

aagggcgaca cggaaatgtt gaatactcat actcttcctt tttcaatatt attgaagcat 3420

ttatcagggt tattgtctca tgagcggata catatttgaa tgtatttaga aaaataaaca 3480

aatagggggtt ccgcgcacat ttccccgaaa agtgccacct gacgtctaag aaaccattat 3540

tatcatgaca ttaacctata aaaataggcg tatcacgagg ccctttcgtc 3590

<210> 15
<211> 3584
<212> DNA
<213> Unknown

<220>
<223> Sequenz stellt eine pflanzliche
Promotor-Terminator-Expressionskassette in Vektor
pUC19 dar

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accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

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tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

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gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

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tttttgtctt ctaaatacat atactaatca actggaaatg taaatatttg ctaatatttc 660

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ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960

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atataatgag gattttgcaa tactttcatt catacacact cactaagttt tacacgatta 1080

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cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc 3360

gacacggaaa tgttgaatac tcatactctt cctttttcaa tattattgaa gcatttatca 3420

gggttattgt ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg 3480

ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc taagaaacca ttattatcat 3540

gacattaacc tataaaaata ggcgtatcac gaggcccttt cgtc 3584

<210> 16
<211> 4507
<212> DNA
<213> Unknown

<220>
<223> Sequenz stellt eine pflanzliche
Promotor-Terminator-Expressionskassette in Vektor
pUC19 dar

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ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

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gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

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ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960

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gcacgttgta aaaaacctga gcatgtgtag ctcagatcct taccgccggt ttcggttcat 1260

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tactgattgt ccgtcgagca aatttacaca ttgccactaa acgtctaaac ccttgtaatt 1380

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aaccatgaca tccacttgga ggatgcaata atgaagaaaa ctacaaattt acatgcaact 1920

agttatgcat gtagtctata taatgaggat tttgcaatac tttcattcat acacactcac 1980

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caatattatt gaagcattta tcagggttat tgtctcatga gcggatacat atttgaatgt 4380

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tttcgtc 4507

<210> 17
<211> 5410
<212> DNA
<213> Unknown

<220>

<223> Sequenz stellt eine pflanzliche Promotor-Terminator-Expressionskassette in Vektor pUC19 dar

<400> 17

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ggattttgca atactttcat tcatacacac tcactaagtt ttacacgatt ataatttctt 180

catagccagc ggatccgata tcgggcccgc tagcgttaac cctgctttaa tgagatatgc 240

gagacgccta tgatcgcatg atatttgctt tcaattctgt tgtgcacgtt gtaaaaaacc 300

tgagcatgtg tagctcagat ccttaccgcc ggtttcggtt cattctaatg aatatatcac 360

ccgttactat cgtattttta tgaataatat tctccgttca atttactgat tgtccgtcga 420

gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

tttatgctaa cgtttgccaa cacttagcaa tttgcaagtt gattaattga ttctaaatta 600

tttttgtctt ctaaatacat atactaatca actggaaatg taaatatttg ctaatatttc 660

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ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960

ggaggatgca ataatgaaga aaactacaaa tttacatgca actagttatg catgtagtct 1020

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Leu Asp Gly Lys Val Ser Gln Gly Val Asn Ala Leu Leu Gly Ser Phe
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Gly Val Glu Leu Thr Asp Thr Pro Thr Thr Lys Gly Leu Pro Leu Val
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Asp Ser Pro Thr Pro Ile Val Leu Gly Val Ser Val Tyr Leu Thr Ile
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gtc att gga ggg ctt ttg tgg ata aag gcc agg gat ctg aaa ccg cgc 11764
Val Ile Gly Gly Leu Leu Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg
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Ala Ser Glu Pro Phe Leu Leu Gln Ala Leu Val Leu Val His Asn Leu
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gct att acc tgg cgg tac tct ctc tgg ggc aat gca tac aat cct aaa   11908
Ala Ile Thr Trp Arg Tyr Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys
            110                 115                 120

cat aaa gag atg gcg att ctg gta tac ttg ttc tac atg tct aag tac   11956
His Lys Glu Met Ala Ile Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr
        125                 130                 135

gtg gaa ttc atg gat acc gtt atc atg ata ctg aag cgc agc acc agg   12004
Val Glu Phe Met Asp Thr Val Ile Met Ile Leu Lys Arg Ser Thr Arg
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caa ata agc ttc ctc cac gtt tat cat cat tct tca att tcc ctc att   12052
Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Ser Leu Ile
155                 160                 165                 170

tgg tgg gct att gct cat cac gct cct ggc ggt gaa gca tat tgg tct   12100
Trp Trp Ala Ile Ala His His Ala Pro Gly Gly Glu Ala Tyr Trp Ser
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gcg gct ctg aac tca gga gtg cat gtt ctc atg tat gcg tat tac ttc   12148
Ala Ala Leu Asn Ser Gly Val His Val Leu Met Tyr Ala Tyr Tyr Phe
            190                 195                 200

ttg gct gcc tgc ctt cga agt agc cca aag tta aaa aat aag tac ctt   12196
Leu Ala Ala Cys Leu Arg Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu
        205                 210                 215

ttt tgg ggc agg tac ttg aca caa ttc caa atg ttc cag ttt atg ctg   12244
Phe Trp Gly Arg Tyr Leu Thr Gln Phe Gln Met Phe Gln Phe Met Leu
    220                 225                 230

aac tta gtg cag gct tac tac gac atg aaa acg aat gcg cca tat cca   12292
Asn Leu Val Gln Ala Tyr Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro
235                 240                 245                 250

caa tgg ctg atc aag att ttg ttc tac tac atg atc tcg ttg ctg ttt   12340
Gln Trp Leu Ile Lys Ile Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe
                255                 260                 265

ctt ttc ggc aat ttt tac gta caa aaa tac atc aaa ccc tct gac gga   12388
Leu Phe Gly Asn Phe Tyr Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly
            270                 275                 280

aag caa aag gga gct aaa act gag tga tctagaaggc ctcctgcttt         12435
Lys Gln Lys Gly Ala Lys Thr Glu
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aatgagatat gcgagacgcc tatgatcgca tgatatttgc tttcaattct gttgtgcacg 12495

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tgaatatatc acccgttact atcgtatttt tatgaataat attctccgtt caatttactg 12615

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                                        Met Val Phe Ala Gly Gly
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gga ctt cag cag ggc tct ctc gaa gaa aac atc gac gtc gag cac att 13378
Gly Leu Gln Gln Gly Ser Leu Glu Glu Asn Ile Asp Val Glu His Ile
        300                 305                 310

gcc agt atg tct ctc ttc agc gac ttc ttc agt tat gtg tct tca act 13426
Ala Ser Met Ser Leu Phe Ser Asp Phe Phe Ser Tyr Val Ser Ser Thr
    315                 320                 325

gtt ggt tcg tgg agc gta cac agt ata caa cct ttg aag cgc ctg acg 13474
Val Gly Ser Trp Ser Val His Ser Ile Gln Pro Leu Lys Arg Leu Thr
330                 335                 340                 345

agt aag aag cgt gtt tcg gaa agc gct gcc gtg caa tgt ata tca gct 13522
Ser Lys Lys Arg Val Ser Glu Ser Ala Ala Val Gln Cys Ile Ser Ala
                350                 355                 360

gaa gtt cag aga aat tcg agt acc cag gga act gcg gag gca ctc gca 13570
Glu Val Gln Arg Asn Ser Ser Thr Gln Gly Thr Ala Glu Ala Leu Ala
            365                 370                 375

gaa tca gtc gtg aag ccc acg aga cga agg tca tct cag tgg aag aag 13618
Glu Ser Val Val Lys Pro Thr Arg Arg Arg Ser Ser Gln Trp Lys Lys
        380                 385                 390

tcg aca cac ccc cta tca gaa gta gca gta cac aac aag cca agc gat 13666
Ser Thr His Pro Leu Ser Glu Val Ala Val His Asn Lys Pro Ser Asp
    395                 400                 405

tgc tgg att gtt gta aaa aac aag gtg tat gat gtt tcc aat ttt gcg 13714
Cys Trp Ile Val Val Lys Asn Lys Val Tyr Asp Val Ser Asn Phe Ala
410                 415                 420                 425

gac gag cat ccc gga gga tca gtt att agt act tat ttt gga cga gac 13762
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Asp Glu His Pro Gly Gly Ser Val Ile Ser Thr Tyr Phe Gly Arg Asp
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ggc aca gat gtt ttc tct agt ttt cat gca gct tct aca tgg aaa att   13810
Gly Thr Asp Val Phe Ser Ser Phe His Ala Ala Ser Thr Trp Lys Ile
            445                 450                 455

ctt caa gac ttt tac att ggt gac gtg gag agg gtg gag ccg act cca   13858
Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu Arg Val Glu Pro Thr Pro
        460                 465                 470

gag ctg ctg aaa gat ttc cga gaa atg aga gct ctt ttc ctg agg gag   13906
Glu Leu Leu Lys Asp Phe Arg Glu Met Arg Ala Leu Phe Leu Arg Glu
    475                 480                 485

caa ctt ttc aaa agt tcg aaa ttg tac tat gtt atg aag ctg ctc acg   13954
Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr Val Met Lys Leu Leu Thr
490                 495                 500                 505

aat gtt gct att ttt gct gcg agc att gca ata ata tgt tgg agc aag   14002
Asn Val Ala Ile Phe Ala Ala Ser Ile Ala Ile Ile Cys Trp Ser Lys
                510                 515                 520

act att tca gcg gtt ttg gct tca gct tgt atg atg gct ctg tgt ttc   14050
Thr Ile Ser Ala Val Leu Ala Ser Ala Cys Met Met Ala Leu Cys Phe
            525                 530                 535

caa cag tgc gga tgg cta tcc cat gat ttt ctc cac aat cag gtg ttt   14098
Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His Asn Gln Val Phe
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gag aca cgc tgg ctt aat gaa gtt gtc ggg tat gtg atc ggc aac gcc   14146
Glu Thr Arg Trp Leu Asn Glu Val Val Gly Tyr Val Ile Gly Asn Ala
    555                 560                 565

gtt ctg ggg ttt agt aca ggg tgg tgg aag gag aag cat aac ctt cat   14194
Val Leu Gly Phe Ser Thr Gly Trp Trp Lys Glu Lys His Asn Leu His
570                 575                 580                 585

cat gct gct cca aat gaa tgc gat cag act tac caa cca att gat gaa   14242
His Ala Ala Pro Asn Glu Cys Asp Gln Thr Tyr Gln Pro Ile Asp Glu
                590                 595                 600

gat att gat act ctc ccc ctc att gcc tgg agc aag gac ata ctg gcc   14290
Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp Ser Lys Asp Ile Leu Ala
            605                 610                 615

aca gtt gag aat aag aca ttc ttg cga atc ctc caa tac cag cat ctg   14338
Thr Val Glu Asn Lys Thr Phe Leu Arg Ile Leu Gln Tyr Gln His Leu
        620                 625                 630

ttc ttc atg ggt ctg tta ttt ttc gcc cgt ggt agt tgg ctc ttt tgg   14386
Phe Phe Met Gly Leu Leu Phe Phe Ala Arg Gly Ser Trp Leu Phe Trp
    635                 640                 645

agc tgg aga tat acc tct aca gca gtg ctc tca cct gtc gac agg ttg   14434
Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu Ser Pro Val Asp Arg Leu
650                 655                 660                 665
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ttg gag aag gga act gtt ctg ttt cac tac ttt tgg ttc gtc ggg aca   14482
Leu Glu Lys Gly Thr Val Leu Phe His Tyr Phe Trp Phe Val Gly Thr
                670                 675                 680

gcg tgc tat ctt ctc cct ggt tgg aag cca tta gta tgg atg gcg gtg   14530
Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro Leu Val Trp Met Ala Val
            685                 690                 695

act gag ctc atg tcc ggc atg ctg ctg ggc ttt gta ttt gta ctt agc   14578
Thr Glu Leu Met Ser Gly Met Leu Leu Gly Phe Val Phe Val Leu Ser
        700                 705                 710

cac aat ggg atg gag gtt tat aat tcg tct aaa gaa ttc gtg agt gca   14626
His Asn Gly Met Glu Val Tyr Asn Ser Ser Lys Glu Phe Val Ser Ala
    715                 720                 725

cag atc gta tcc aca cgg gat atc aaa gga aac ata ttc aac gac tgg   14674
Gln Ile Val Ser Thr Arg Asp Ile Lys Gly Asn Ile Phe Asn Asp Trp
730                 735                 740                 745

ttc act ggt ggc ctt aac agg caa ata gag cat cat ctt ttc cca aca   14722
Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro Thr
                750                 755                 760

atg ccc agg cat aat tta aac aaa ata gca cct aga gtg gag gtg ttc   14770
Met Pro Arg His Asn Leu Asn Lys Ile Ala Pro Arg Val Glu Val Phe
            765                 770                 775

tgt aag aaa cac ggt ctg gtg tac gaa gac gta tct att gct acc ggc   14818
Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Ile Ala Thr Gly
        780                 785                 790

act tgc aag gtt ttg aaa gca ttg aag gaa gtc gcg gag gct gcg gca   14866
Thr Cys Lys Val Leu Lys Ala Leu Lys Glu Val Ala Glu Ala Ala Ala
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Glu Gln His Ala Thr Thr Ser
810                 815

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cctgagcatg tgtagctcag atccttaccg ccggtttcgg ttcattctaa tgaatatatc 15040

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Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
         35                  40                  45

Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
     50                  55                  60

Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
 65                  70                  75                  80

Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                 85                  90                  95

Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110

Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125

Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140

Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160

Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175

His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190

Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205

Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
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Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240

Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255

Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
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Ser Tyr Val Ser Ser Thr Val Gly Ser Trp Ser Val His Ser Ile Gln
        35                  40                  45

Pro Leu Lys Arg Leu Thr Ser Lys Lys Arg Val Ser Glu Ser Ala Ala
    50                  55                  60

Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
65                  70                  75                  80

Thr Ala Glu Ala Leu Ala Glu Ser Val Val Lys Pro Thr Arg Arg Arg
                85                  90                  95

Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
            100                 105                 110

His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
        115                 120                 125

Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
    130                 135                 140

Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
145                 150                 155                 160

Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
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Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
            180                 185                 190

Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
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Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
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Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
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Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
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Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
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Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
        275                 280                 285

Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
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Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
305                 310                 315                 320

Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
                325                 330                 335

Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
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Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
        355                 360                 365

Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
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Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
385                 390                 395                 400

Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
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Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
            420                 425                 430

Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
        435                 440                 445

Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu
    450                 455                 460

His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Lys Ile Ala
465                 470                 475                 480

Pro Arg Val Glu Val Phe Cys Lys Lys His Gly Leu Val Tyr Glu Asp
                485                 490                 495

Val Ser Ile Ala Thr Gly Thr Cys Lys Val Leu Lys Ala Leu Lys Glu
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Leu Asp Gly Lys Val Ser Gln Gly Val Asn Ala Leu Leu Gly Ser Phe
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Gly Val Glu Leu Thr Asp Thr Pro Thr Thr Lys Gly Leu Pro Leu Val
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gac agt ccc aca ccc atc gtc ctc ggt gtt tct gta tac ttg act att  11716
Asp Ser Pro Thr Pro Ile Val Leu Gly Val Ser Val Tyr Leu Thr Ile
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Val Ile Gly Gly Leu Leu Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg
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Ala Ser Glu Pro Phe Leu Leu Gln Ala Leu Val Leu Val His Asn Leu
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Phe Cys Phe Ala Leu Ser Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln
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Ala Ile Thr Trp Arg Tyr Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys
            110                 115                 120

cat aaa gag atg gcg att ctg gta tac ttg ttc tac atg tct aag tac  11956
His Lys Glu Met Ala Ile Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr
        125                 130                 135

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Val Glu Phe Met Asp Thr Val Ile Met Ile Leu Lys Arg Ser Thr Arg
    140                 145                 150

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Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Ser Leu Ile
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Ala Ala Leu Asn Ser Gly Val His Val Leu Met Tyr Ala Tyr Tyr Phe
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Leu Ala Ala Cys Leu Arg Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu
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Gln Trp Leu Ile Lys Ile Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe
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Leu Phe Gly Asn Phe Tyr Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly
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                                                295

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gcc agt atg tct ctc ttc agc gac ttc ttc agt tat gtg tct tca act   13426
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Ser Lys Lys Arg Val Ser Glu Ser Ala Ala Val Gln Cys Ile Ser Ala
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Glu Val Gln Arg Asn Ser Ser Thr Gln Gly Thr Ala Glu Ala Leu Ala
            365                 370                 375

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Glu Ser Val Val Lys Pro Thr Arg Arg Arg Ser Ser Gln Trp Lys Lys
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Ser Thr His Pro Leu Ser Glu Val Ala Val His Asn Lys Pro Ser Asp
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Cys Trp Ile Val Val Lys Asn Lys Val Tyr Asp Val Ser Asn Phe Ala
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Asp Glu His Pro Gly Gly Ser Val Ile Ser Thr Tyr Phe Gly Arg Asp
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Gly Thr Asp Val Phe Ser Ser Phe His Ala Ala Ser Thr Trp Lys Ile
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Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu Arg Val Glu Pro Thr Pro
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Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr Val Met Lys Leu Leu Thr
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Asn Val Ala Ile Phe Ala Ala Ser Ile Ala Ile Ile Cys Trp Ser Lys
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Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His Asn Gln Val Phe
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His Ala Ala Pro Asn Glu Cys Asp Gln Thr Tyr Gln Pro Ile Asp Glu
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Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp Ser Lys Asp Ile Leu Ala
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Thr Val Glu Asn Lys Thr Phe Leu Arg Ile Leu Gln Tyr Gln His Leu
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Phe Phe Met Gly Leu Leu Phe Phe Ala Arg Gly Ser Trp Leu Phe Trp
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Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu Ser Pro Val Asp Arg Leu
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Thr Glu Leu Met Ser Gly Met Leu Leu Gly Phe Val Phe Val Leu Ser
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His Asn Gly Met Glu Val Tyr Asn Ser Ser Lys Glu Phe Val Ser Ala
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cag atc gta tcc aca cgg gat atc aaa gga aac ata ttc aac gac tgg   14674
Gln Ile Val Ser Thr Arg Asp Ile Lys Gly Asn Ile Phe Asn Asp Trp
730                 735                 740                 745

ttc act ggt ggc ctt aac agg caa ata gag cat cat ctt ttc cca aca   14722
Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro Thr
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Met Pro Arg His Asn Leu Asn Lys Ile Ala Pro Arg Val Glu Val Phe
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Arg Gln Arg Gln Thr Thr Ala Val Ala Lys His Asn Ala Ala Thr Ile
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Ser Thr Gln Glu Arg Leu Cys Ser Leu Ser Ser Leu Lys Gly Glu Glu
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gtc tgc atc gac gga atc atc tat gac ctc caa tca ttc gat cat ccc   15958
Val Cys Ile Asp Gly Ile Ile Tyr Asp Leu Gln Ser Phe Asp His Pro
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ggg ggt gaa acg atc aaa atg ttt ggt ggc aac gat gtc act gta cag   16006
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Thr Glu Phe Glu Arg Glu Ile Lys Arg Glu Val Phe Lys Ile Val Arg
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Thr Ser Trp Leu Leu Ala Val Ala Tyr Gly Ile Ser Gln Ala Met Ile
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Gly Met Asn Val Gln His Asp Ala Asn His Gly Ala Thr Ser Lys Arg
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Pro Trp Val Asn Asp Met Leu Gly Leu Gly Ala Asp Phe Ile Gly Gly
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Asn His Ala Glu Met Asp Pro Asp Ser Phe Gly Ala Glu Pro Met Leu
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Leu Phe Asn Asp Tyr Pro Leu Asp His Pro Ala Arg Thr Trp Leu His
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Arg Phe Gln Ala Phe Phe Tyr Met Pro Val Leu Ala Gly Tyr Trp Leu
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Electronic Patent Application Fee Transmittal

Application Number:	15256914
Filing Date:	06-Sep-2016
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Filer:	Hui-Ju Wu/jamie jensen-smith
Attorney Docket Number:	074017-0013-01-US

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	30745689
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Hui-Ju Wu/jamie jensen-smith
Filer Authorized By:	Hui-Ju Wu
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	24-OCT-2017
Filing Date:	06-SEP-2016
Time Stamp:	15:40:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$180
RAM confirmation Number	102517INTEFSW15413700
Deposit Account	500573
Authorized User	Jamie Jensen-Smith

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	SupplementalInformationDisclosureStatement.pdf	27856 4182e278345e6c92c77fa1674581ee6cdef74095	no	2
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	SB08.pdf	43827 5a3e55bcca1de9f33257b988d6198e8c5b068465	no	2
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3	Foreign Reference	WO02057465A2.pdf	14084561 ec1bdfc103aed5c16dc776936af3c39c45145332	no	224
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Warnings:					
Information:					
6	Non Patent Literature	MICHAELSON_1998_215.pdf	195693 57c6a86dc1ffaf4490475be1a68a879b18532cc7	no	4
Warnings:					

Information:					
7	Non Patent Literature	MICHAELSON_1998_19055.pdf	407110 1d0042ea978bcebfd3df03d1dd94bf828571c144	no	6
Warnings:					
Information:					
8	Non Patent Literature	MOON_2001_45358.pdf	513318 8c052d1617d2cd840a30c60db9180c0768f49653	no	10
Warnings:					
Information:					
9	Non Patent Literature	Sayanova_1997_4211.pdf	376401 2a2db428f431cf6360469fa1577d8d2546eb9480	no	6
Warnings:					
Information:					
10	Non Patent Literature	Shahidi_Bailey.pdf	59782 84f236456fb9be76adcec6123ed087833971df00	no	2
Warnings:					
Information:					
11	Non Patent Literature	Sperling_2000_3801.pdf	1310295 c9862ed926a5518280a47fc40120b785bad5fbbf	no	11
Warnings:					
Information:					
12	Non Patent Literature	Tonon_2003_440.pdf	424283 96b2d6550db20f0ff76fd1b43b3b71fd6bf4de3f	no	5
Warnings:					
Information:					
13	Non Patent Literature	CODEX.pdf	202023 1ed32ff80869f0df8fe82704c6eb0db8622050a8	no	15
Warnings:					
Information:					

14	Non Patent Literature	Watts_1999_175.pdf	617310 bf4f4bd7b485001134805 4b5ee4cc78c55ff88ce	no	8
Warnings:					
Information:					
15	Non Patent Literature	Girke.pdf	873321 53292a6ee303c9a670efd8d2c9722515dcbd63c3	no	10
Warnings:					
Information:					
16	Fee Worksheet (SB06)	fee-info.pdf	30879 adcc33b258beb9ff8f1a98913cc1d6829defea94	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	19362785	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 074017-0013-01-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: Hope A. Robinson

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 CFR 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed after the mailing date of the first Office Action on the merits, but before the mailing date of any of a Final Office Action, a Notice of Allowance (37 CFR 1.97(c)) or an action that otherwise closes prosecution in the application.

In accordance with 37 CFR 1.98(a)(2)(ii), Applicant has not submitted copies of U.S. patent applications cited in the attached PTO/SB/08. Applicant submits herewith copies of foreign patents and non-patent literature in accordance with 37 CFR 1.98(a)(2).

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this

Information Disclosure statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such. Moreover, Applicant understands that the Examiner will make an independent evaluation of the cited documents.

Applicant notes that the references listed in the translation column of the attached Form PTO SB/08 are English-language counterparts from the same family of applications as the non - English foreign patent documents listed therein, but may not necessarily be their English-language equivalents.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the listed references.

Accompanying this submission is the payment in the amount of $180.00 covering the fee set forth in 37 C.F.R. § 1.17(p). Applicant believes no further fee is due. However, if any additional fee is due, the Director is hereby authorized to charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US, from which the undersigned is authorized to draw.

Respectfully submitted,

By___/ *Hui-Ju Wu* /_____

Hui-Ju Wu, Ph.D.
Registration No.: 57,209
Drinker Biddle & Reath LLP
222 Delaware Ave., Ste. 1410
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(302) 351-6938 (Fax)
Attorney for Applicant

#90,345,755

Electronic Acknowledgement Receipt

EFS ID:	30745882
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Hui-Ju Wu/jamie jensen-smith
Filer Authorized By:	Hui-Ju Wu
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	24-OCT-2017
Filing Date:	06-SEP-2016
Time Stamp:	15:47:35
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Kang.pdf	440924 60f12519a780720f50db43678c01571c138fedde	no	5

Warnings:

Information:	
Total Files Size (in bytes):	440924

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 01/30/2018

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
01/30/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

IPDocketWM@dbr.com
penelope.mongelluzzo@dbr.com
DBRIPDocket@dbr.com

Office Action Summary	Application No. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10/23/17*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-23* is/are pending in the application.

5a) Of the above claim(s) *6,11,12,15 and 17-22* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-5,7-10,13,14,16 and 23* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Application Status

1. The present application is being examined under the pre-AIA first to invent provisions.

2. The Amendment filed on October 23, 2017, has been received and entered.

Claim Disposition

3. Claims 1-23 are pending. Claims 1-5, 7-10, 13-14, 16 and 23 are under examination. Claims 6, 11-12, 15 and 17-22 are withdrawn from further consideration pursuant to 37 CFR 1.12(b), as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Information Disclosure Statement

4. The Information Disclosure Statement filed on October 24, 2017 has been received and entered. The references cited on the PTO-1449 Form have been considered by the examiner and a copy is attached to the instant Office action.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to

consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

6. Claims 1-5, 7-10, 13-14, 16 and 23 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being obvious over Cirpus et al. (WO2005/083093, 9/9/05 with US Patent No.9458436, 2004 as English equivalence, of record in the application).

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under pre-AIA 35 U.S.C. 102(e). This rejection under pre-AIA 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention “by another”; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131(a); or (3) an oath or declaration under 37 CFR 1.131(c) stating that the application and reference are currently owned by the same party and that the inventor or joint inventors (i.e., the inventive entity) named in the application is the prior inventor under pre-AIA 35 U.S.C. 104 as in effect on March 15, 2013, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under pre-AIA 35 U.S.C. 103(c) as prior art in a rejection under pre-AIA 35 U.S.C. 103(a). See MPEP §§ 706.02(l)(1) and 706.02(l)(2).

The present invention relates to oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant and comprise certain weight percent of EPA, DHA and DPA (see instant claim 1 for example).

The cited reference invention is directed to a process for the production of polyunsaturated fatty acids in the seed of transgenic plants by introducing, into the organism, nucleic acids which encode polypeptides with an .omega.3-desaturase, .DELTA.12-desaturase, .DELTA.6-desaturase, .DELTA.6-elongase, .DELTA.5-desaturase, .DELTA.5-elongase and/or .DELTA.4-desaturase activity. The invention of the published document further relates to recombinant nucleic acid molecules comprising the nucleic acid sequences which encode the aforementioned polypeptides, either jointly or individually, and transgenic plants which comprise the aforementioned recombinant nucleic acid molecules. Furthermore, the invention in the art relates to the generation of a transgenic plant and to oils, lipids and/or fatty acids with an elevated content of polyunsaturated fatty acids, in particular arachidonic acid, eicosapentaenoic acid and/or docosahexaenoic acid, as the result of the expression of the elongases and desaturases used in the process according to the invention (see abstract of WO document; and abstract of patent with paragraph 6 of the patent).

The WO 2005/083093 document discloses products comprising at least 16% by weight of EPA (see page 26, lines 14-18 of WO 2005/083093) and at least 0.35% by weight of DHA (see page 26, lines 20-24 of WO 2005/083093). Additionally, Table 24 on page 165 of WO 2005/083093 shows that transgenic seeds had an EPA content of 8.65 to 9.43%, a DPA content of 0.19 to 0.24% and a DHA content of 0.23 to 0.40%.

Additionally, Tables 22 and 23 on page 158 of WO 2005/083093 disclose that transgenic seeds showed an EPA content of 0.2 to 2.6% based on the total fatty acids. Further, Table 3 on pages 161 and 162 of WO 2005/083093 depicts that seeds from transgenic *B. juncea* had an EPA content of 0.94 to 1.21% and Table 4 on page 163 shows that transgenic *B. juncea* had an EPA content of 0.8 to 2.6%. Moreover, the patent equivalence discloses at paragraph 89 of the patent it is disclosed that, “DHA is produced in the process according to the invention in a content of at least 0.01 or 0.02% by weight, advantageously at least 0.03 or 0.05% by weight, advantageously at least 0.09 or 0.1% by weight especially preferably at least 0.2 or 0.3% by weight and most preferably at least 0.35% by weight based on the total lipid content in the seeds of the transgenic plants. At paragraph 8 of the patent it is disclosed that there is a higher content of LCPUFAs. DHA in the form of triacylglycerides is disclosed (see paragraph 17, for example). The weight percent recited in for example claim 9 is obvious based on the disclosure in paragraphs 59-80.

Therefore, it would have been obvious to one of ordinary skill in the art to arrive at the claimed invention as a whole based on the teachings in the cited reference. Although the EPA content disclosed in WO 2005/083093 is lower than the EPA content required by the present claims, the cited published WO document discloses that the invention pertains to production of transgenic plant or organism with an increased fatty acid content and thus an ordinary skilled worker in the field would know how to perform routine experimentation (see MPEP 2144.05) to optimize and obtain a higher weight percentage of each of EPA, DHA and DPA having the specific transgenic plant or

organism to produce them based on the teaching in the cited art. The skilled artisan would optimize the amount routinely since this would be an expected result. "Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454,456, 105 USPQ 233, 245 (CCPA 1955)."

Response to Arguments

7. Applicant's comments have been considered in full. Withdrawn objections/rejections will not be discussed herein as applicant's comments are moot. The rejection for record under 35 USC 102 is withdrawn and replaced with a 103 rejection; and applicant's statements regarding the relationship between the patent and the WO document are correct. Based on the established equivalency applicant's discussion of the reference pertaining to the claimed invention, it has been added and will be discussed herein. Applicant traverses the application of the art over the instant invention stating that, the Examiner asserts that Cirpus discloses the production of polyunsaturated fatty acids in the seeds of transgenic plants by expressing nucleic acid sequences which increase the content of polyunsaturated fatty acids as well as oils,

lipids and/or fatty acids with an elevated content of polyunsaturated fatty acids. The Examiner further alleges that the percentage for DHA disclosed in Cirpus corresponds to that recited in the claims, applicant strongly disagrees. Applicant acknowledges that the cited reference is pertinent and tries to attack the reference that has a common inventor by stating that there is a percentage of for example 16% in the art with claim 1 requiring 20% weight content. The rejection has been cited under 35 USC 103 for that very reason, the art remains relevant because an ordinary skilled worker in the field would know how to optimize the expected results to attain a higher weight percentage. Note that the cited art discloses the same method in the same transgenic plant for the same reason to increase fatty acid content. The percentage achieved in the references as a trial does not inhibit a higher percentage and would be expected and routine with optimization. Applicant's arguments are not persuasive because routine optimization is an obvious matter of choice based on the teaching of the reference to increase fatty acid content and is of expected results. An express teaching/suggestion of this is not necessary to render obviousness (see MPEP 2144.06 II).Thus, the claimed invention is *prima facie* obvious. Based on the clarification provided and the newly instituted rejection the action is made non-final.

Conclusion

8. No claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday from 9:00 a.m. to 5:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi, can be reached at (571) 272-0956.

The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Hope A. Robinson/

Primary Examiner, Art Unit 1652

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /H.A.R/

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	15/256,914 – Conf.#4050
				Filing Date	September 6, 2016
				First Named Inventor	Petra CIRPUS
				Art Unit	1652
(Use as many sheets as necessary)				Examiner Name	Hope A. Robinson
Sheet	1	of	2	Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA*	US-6,194,167-B1	2001-02-27	Browse et al.	
	AB*	US-7,211,656-B2	2007-05-01	Mukerji et al.	
	AC*	US-7,238,851-B2	2007-07-03	Kang	
	AD*	US-8,049,064-B2	2011-11-01	Cirpus et al.	
	AE*	US-8,088,974-B2	2012-01-03	Lerchl et al.	
	AF*	US-8,455,035-B2	2013-06-04	Rein et al.	
	AG*	US-8,993,841-B2	2015-03-31	Napier et al.	
	AH*	US-8,785,727-B2	2014-07-22	Bauer et al.	
	AI*	US-9,493,520-B2	2016-11-15	Bauer et al.	
	AJ*	US-2003/0163845-A1	2003-08-28	Mukerji et al.	
	AK*	US-2003/0196217-A1	2003-10-16	Mukerji et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BA	WO-02/057465-A2	2002-07-25	BASF Plant Science GmbH		See US 8,088,974

Examiner Signature	/HOPE A ROBINSON/	Date Considered	01/25/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /H.A.R/

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	15/256,914 – Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	"Codex Standard for Named Vegetable Oils – CX-STAN 210-1999", excerpt from Codex Alimentarius, 2001, Vol. 8, pp. 11-25.	
	CB	"Danio rerio Polyunsaturated Fatty Acid Elongase mRNA, Complete cds", Database GenBank, Accession No. AF532782, February 15, 2006.	
	CC	"Phaeodactylum tricornutum Delta 12 Fatty Acid Desaturase mRNA, Complete cds; Nuclear Gene for Microsomal Protein", Database GenBank, Accession No. AY165023, April 14, 2003.	
	CD	GIRKE, T., et al., "Identification of a Novel Δ6-Acyl-Group Desaturase by Targeted Gene Disruption in Physcomitrella patens", The Plant Journal, 1998, Vol. 15, No. 1, pp. 39-48.	
	CE	MICHAELSON, L. V., et al., "Functional Identification of a Fatty Acid Δ5 Desaturase Gene from Caenorhabditis elegans", FEBS Letters, 1998, Vol. 439, No. 3, pp. 215-218.	
	CF	MICHAELSON, L. V., et al., "Isolation of a Δ5-Fatty Acid Desaturase Gene from Mortierella alpina", The Journal of Biological Chemistry, 1998, Vol. 273, No. 30, pp. 19055-19059.	
	CG	MOON, Y.-A., et al., "Identification of a Mammalian Long Chain Fatty Acyl Elongase Regulated by Sterol Regulatory Element-Binding Proteins", The Journal of Biological Chemistry, 2001, Vol. 276, No. 48, pp. 45358-45366.	
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	CI	"Future Considerations", page 221 of "Bailey's Industrial Oil and Fat Products", Sixth Edition, Vol. 6, SHAHIDI, F., Ed., John Wiley & Sons, Inc., 2005.	
	CJ	SPERLING, P., et al., "A Bifunctional Δ6-Fatty Acyl Acetylenase/Desaturase from the Moss Ceratodon purpureus", European Journal of Biochemistry, 2000, Vol. 267, No. 12, pp. 3801-3811.	
	CK	TONON, T., et al., "Identification of a Very Long Chain Polyunsaturated Fatty Acid Δ4-Desaturase from the Microalga Pavlova lutheri", FEBS letters, 2003, Vol. 553, No. 3, pp. 440-444.	
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	CM	KANG, Z. B., et al., "Adenoviral Gene Transfer of *Caenorhabditis elegans* n-3 Fatty Acid Desaturase Optimizes Fatty Acid Composition in Mammalian Cells", PNAS, 2001, Vol. 98, No. 7, pp. 4050-4054.	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	01/25/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

#90,345,659

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	Art Unit
	HOPE ROBINSON	1652

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
NONE		1/25/18	HAR

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

SEARCH NOTES		
Search Notes	Date	Examiner
Palm Expo inventor names searched	1/25/18	HAR
NPL search (DHA, transgeic Brassica plant, oils, lipids and fatty acids, LCPUFA, triacylglycerides)	1/25/18	HAR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

Docket No.: 074017-0013-01-US-541474
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: H. A. Robinson

AMENDMENT UNDER 37 C.F.R. § 1.111

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Amendment responds to the Office Action dated January 30, 2018, a response date to which extends through May 30, 2018, in view of the accompanying Petition for one-month Extension of Time and associated fee. Please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper; and

Remarks/Arguments begin on page 8 of this paper.

AMENDMENTS TO THE CLAIMS

Please amend the claims of the above-identified application as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended): Oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant, wherein said polyunsaturated fatty acids comprise at least 20% by weight of eicosapentaenoic acid (EPA), at least 2% by weight of docosapentaenoic acid (DPA), ~~and/or~~ and at least 4% by weight of docosahexaenoic acid (DHA) based on the total fatty acids in the transgenic plant in form of triacylglycerides.

2. (Currently Amended): The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-1, sn-2 or sn-3 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 1.5% by weight of DPA is present in the sn-1, sn-2 or sn-3 position; ~~and/or~~ and

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-1, sn-2 or sn-3 position.

3. (Currently Amended): The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at

least 24% by weight of EPA is present in the sn-2 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 3% by weight of DPA is present in the sn-2 position; ~~and/or~~ and

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-2 position.

4. (Original): The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise:

a) at least 20% by weight of EPA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides:

b) at least 20% by weight of EPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides; or

c) at least 2% by weight of DPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

5. (Original): The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

6. (Withdrawn): The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides

based on the total fatty acids in the transgenic plant.

7. (Original): The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

8. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

9. (Previously Presented): Oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

10. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant.

11. (Withdrawn): The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 20% by weight of EPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

12. (Withdrawn): The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

13. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in form of triacylglycerides.

14. (Original): The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

15. (Withdrawn): The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

16. (Original): The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in form of triacylglycerides based on the total fatty acids in the transgenic plant.

17. (Withdrawn): A method for producing oils, lipids and/or fatty acids of claim 1,

comprising expressing in a *Brassica* plant a nucleic acid encoding a Δ6-desaturase, a nucleic acid encoding a Δ5-desaturase, a nucleic acid encoding a Δ6-elongase, a nucleic acid encoding a ω3-desaturase, a nucleic acid encoding a Δ5-elongase, and a nucleic acid encoding a Δ4-desaturase, wherein said nucleic acid encoding a Δ5-elongase is codon-optimized by adapting to the codon usage of *Brassica*.

18. (Withdrawn): The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a nucleotide sequence having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 64, and wherein said nucleotide sequence is obtained by adapting at least 30% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of *Brassica*.

19. (Withdrawn): The method of claim 18, wherein said nucleotide sequence has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 64, or wherein said nucleotide sequence encodes a polypeptide having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65.

20. (Withdrawn): The method of claim 18, wherein said nucleotide sequence is adapted taking into account the natural frequency of individual codons.

21. (Withdrawn): The method of claim 18, wherein said nucleotide sequence is

obtained by adapting at least 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of *Brassica*.

22. (Withdrawn): The method of claim 17, wherein said nucleic acid encoding a Δ5-elongase comprises a translated section coding for a protein having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65, and wherein the translated section comprises a nucleotide sequence obtained by adapting at least 30% or 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of *Brassica*.

23. (Previously presented): The oils, lipids and/or fatty acids of claim 1, comprising:

a) at least 4% by weight of DHA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant;

b) at least 4% by weight of DHA and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant; or

c) at least 4% by weight of DHA, at least 2% by weight of DPA, and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant.

REMARKS

Entry of the foregoing amendments and reconsideration of the subject application are respectfully requested in light of the comments which follow.

1. Status of the Claims

Claims 1-23 are pending. Claims 1-3 are amended to replace "and/or" with "and." Claims 6, 11, 12, 15, and 17-22 stand withdrawn.

Claims 1-5, 7-10, 13, 14, 16, and 23 are under examination.

2. Acknowledgement of Information Disclosure Statement

Applicant notes with appreciation the acknowledgment of the Information Disclosure Statement (IDS) filed October 24, 2017.

Applicant respectfully requests consideration of the IDS accompanying this response.

3. Rejection of the Claims Under 35 U.S.C. § 103(a)

Claims 1-5, 7-10, 13-14, 16, and 23 stand rejected under pre-AIA 35 U.S.C. § 103(a) as being obvious over Cirpus et al. (WO 2005/083093, 9/9/05 with US Patent No. 9,458,436, as English equivalence, of record in the application) (hereinafter "*Cirpus*"), for the reasons set forth on pages 5-7 of the Office Action. The Examiner has maintained the rejection, asserting that the ordinary skilled worker would know how to perform routine experimentation to optimize and obtain a higher percentage of each of EPA, DHA and DPA.

Applicant traverses the rejection to the extent it may be applied to the amended claims. To establish a *prima facie* case of obviousness, all the claim limitations must be taught or suggested by the prior art. See *In re Royka*, 490 F.2d 981, 985, 180 USPQ 580 (CCPA 1974); *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1342, 68 U.S.P.Q.2d 1940, 1947 (Fed. Cir. 2003). In determining the differences between the prior art and the claims under the Graham analysis, the invention as a whole must be considered (MPEP 2141.02; citing *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983)). Additionally, once the scope and content of the prior art are determined, the relevant inquiry is whether the prior art suggests the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful. *In re Vaeck*, 947 F.2d 488, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991).

Claim 1

Claim 1 as amended is directed to oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant, wherein said polyunsaturated fatty acids comprise at least 20% by weight of eicosapentaenoic acid (EPA), at least 2% by weight of docosapentaenoic acid (DPA), ***and*** at least 4% by weight of docosahexaenoic acid (DHA) based on the total fatty acids in the transgenic plant in form of triacylglycerides. Accordingly, amended claim 1 requires the presence of each of the recited components in the recited amounts.

Cirpus is directed to increasing the amount of ARA, EPA, and DHA in a transgenic plant. *Cirpus* offers detailed disclosures about the weight percentage for each of ARA, EPA, and DHA. *See*, for instance, col. 22, lines 5-25. Notably, however, *Cirpus* is does not provide any detailed disclosure on the content of DPA. For instance, when discussing mixtures of different polyunsaturated C20- and/or C22-fatty acids in col. 22, lines 56-64, *Cirpus* discloses mixtures of DHA with ARA and/or EPA, but is silent regarding DPA. One disclosure in *Cirpus* specific to the weight percent of DPA is found in Table 24, which discloses data for seeds of three transgenic Indian Mustard plants. Table 24 discloses a DPA ranging from 0.19 to 0.24%. This range does not overlap the recited amount of at least 2% by weight of DPA. Indeed, the disclosed range is *an order of magnitude less* than the recited amount of DPA, and thus is not even close to the DPA amount recited in amended claim 1. *Cirpus* does not teach or suggest the recited amount of DPA. Moreover, *Cirpus* does not teach the skilled person how to obtain a composition having the claimed content of DPA. For at least this reason, the rejection should be withdrawn.

Additionally, *Cirpus* discloses that fatty acid mixtures produced by the process of the *Cirpus* disclosure should contain virtually no DPA. Specifically, in col. 23, lines 56-62, *Cirpus* teaches: "The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1%, based on the total fatty acids, or ....no clupanodonic acid (=docosapentaenoic acid, $C22:5^{\Delta 4,8,12,15,21}$)..." This disclosure would have clearly guided the skilled artisan *away* from a composition comprising more than 0.1% DPA, and certainly would have guided the skilled artisan away from a composition comprising at least 2% by weight of DPA.

For at least these reasons, claim 1, and claims dependent thereon, are novel and nonobvious over *Cirpus*.

Claim 9

Claim 9 is directed to oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

The Examiner asserts that this weight percent is obvious based on paragraphs 59-80 of *Cirpus*. Applicant notes that neither WO 2005/083093 nor US Patent No. 9,458,436 (English equivalence of WO 2005/0830) includes paragraph numbers. Applicant therefore respectfully requests clarification as to what disclosure the Examiner is referring.

Applicant further notes that according to Table 24 in *Cirpus*, the sum of all polyunsaturated ω3-fatty acids ranges from 14.58% to 15.72%. This range does not overlap the recited amount of at least 54% by weight of polyunsaturated ω3-fatty acids. Indeed, the disclosed range is much less than the recited amount of polyunsaturated ω3-fatty acids, and thus not even close to the polyunsaturated ω3-fatty acids amount recited in amended claim 9. *Cirpus* does not teach or suggest the recited amount of polyunsaturated ω3-fatty acids. Moreover, *Cirpus* does not teach the skilled person how to obtain a composition having the claimed content of polyunsaturated ω3-fatty acid. The skilled artisan would not have been guided to use *Brassica* plants to produce oils, lipids and fatty acids with the claimed content of polyunsaturated ω3-fatty acids.

For at least this reason, the rejection of claim 9, and claims dependent thereon, should be withdrawn.

Not Routine Optimization

The Examiner has alleged that the person of ordinary skill in the art knows how to perform routine experimentation to optimize and obtain a higher percentage of each of EPA, DHA and DPA. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). That is not the case with the present rejection. Optimization within the prior art conditions, for instance, within the range of 0.19 to 0.24% DPA and 14.58 to 15.72% omega3-fatty acids disclosed by *Cirpus*, may be routine optimization. However, it is *not routine optimization* to extend outside of the range disclosed by the prior art. See, for instance, *Peterson*, 315 F.3d at 1330, 65 USPQ2d at 1382 and *In re Hoeschele*, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969). As discussed above, amended claim 1 recites at least 2% by weight of docosapentaenoic acid (DPA). This range is does not overlap, and is not even close to, the disclosure in the applied art. Similarly, claim 9 recites a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids. This range does not overlap the 14.58% to 15.72% range disclosed in the applied art regarding polyunsaturated ω3-fatty acids content. Thus, there is no legal basis for alleging the claimed range for at least DPA in claim 1 and the claimed range of polyunsaturated ω3-fatty acids in claim 9 could arise from routine optimization.

Cirpus does not support *prima facie* obviousness of the amended claims for at least these reasons. Withdrawal of the rejection over *Cirpus* is requested.

CONCLUSIONS

In view of the foregoing, Applicant(s) submit(s) that the pending claims are in condition for allowance, and respectfully request(s) reconsideration and timely allowance of the pending claims. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact Applicants' undersigned representative to expedite prosecution. A favorable action is awaited.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Dated: May 30, 2018

Respectfully submitted,

Customer Number: 123223

By _____
Bronwen M. Loeb, Ph.D.
Registration No.: 43,516
DRINKER BIDDLE & REATH LLP
222 Delaware Avenue, Ste 1410
Wilmington, Delaware 19801
202.230.5438 (Phone)
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Attorneys/Agents For Applicant

PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 074017-0013-01-US-541474
Application Number 15/256,914	Filed September 6, 2016
For METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS	
Art Unit 1652	Examiner H. A. Robinson

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
[X] One month (37 CFR 1.17(a)(1))	$200	$100	$50	$ 200.00
[ ] Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$
[ ] Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
[ ] Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

[ ] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[X] Payment by credit card. Form PTO-2038 is attached.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 50-0573 .

[X] Payment made via EFS-Web.

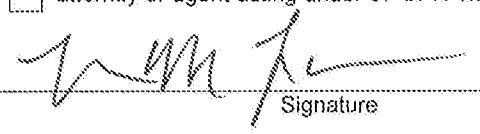
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant.

[X] attorney or agent of record. Registration number 43,516 .

[ ] attorney or agent acting under 37 CFR 1.34. Registration number _____ .



Signature

May 30, 2018
Date

Bronwen M. Loeb, Ph.D.
Typed or printed name

202.230.5438
Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[X] * Total of 1 forms are submitted.

Docket No.: 074017-0013-01-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: Hope A. Robinson

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 CFR 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed after the mailing date of the first Office Action on the merits, but before the mailing date of any of a Final Office Action, a Notice of Allowance (37 CFR 1.97(c)) or an action that otherwise closes prosecution in the application.

In accordance with 37 CFR 1.98(a)(2)(ii), Applicant has not submitted copies of U.S. patent applications cited in the attached PTO/SB/08. Applicant submits herewith copies of foreign patents and non-patent literature in accordance with 37 CFR 1.98(a)(2).

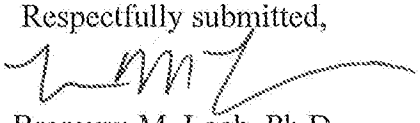
In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this

Information Disclosure statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such. Moreover, Applicant understands that the Examiner will make an independent evaluation of the cited documents.

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98 and the Examiner is respectfully requested to consider the listed references.

Accompanying this submission is the payment in the amount of $240.00 covering the fee set forth in 37 C.F.R. § 1.17(p). Applicant believes no further fee is due. However, if any additional fee is due, the Director is hereby authorized to charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US, from which the undersigned is authorized to draw.

Respectfully submitted,



Dated: May 30, 2018

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PTO/SB/08b (07-09)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	15/256,914 -- Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20150361404	12-17-2015	BASF Plant Science GmbH	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	WAGNER, et al., "Generation of glycerophospholipid molecular species in the yeast Saccharomyces cerevisiae. Fatty acid pattern of phospholipid classes and selective acyl turnover at sn-1 and sn-2 positions", Yeast, Vol. 10, 1994, pp. 1429-1437	
	CB	DIEDRICH, et al., "The natural occurrence of unusual fatty acids. Part 1. Odd numbered fatty acids", Molecular Nutrition & Food Research, Vol. 34, Issue 10, 1990, pp. 935-943	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Electronic Patent Application Fee Transmittal

Application Number:	15256914
Filing Date:	06-Sep-2016
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Filer:	Bronwen M. Loeb
Attorney Docket Number:	074017-0013-01-US

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	1251	1	200	200
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
		Total in USD ($)		**440**

Electronic Acknowledgement Receipt

EFS ID:	32757276
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Bronwen M. Loeb
Filer Authorized By:	
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	30-MAY-2018
Filing Date:	06-SEP-2016
Time Stamp:	14:07:35
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$440
RAM confirmation Number	053118INTEFSW14100400
Deposit Account	500573
Authorized User	Bronwen Loeb

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Amendment.PDF	6497418 25817445225d8f0a4babcf640c6aec56baab9428	yes	13

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	7
	Applicant Arguments/Remarks Made in an Amendment	8	13

Warnings:

Information:

2	Extension of Time	EOT.PDF	762662 795f72d0d3606038e1111aa7d7596a98478d81b0	no	1

Warnings:

Information:

3		IDS_SB08.PDF	2020636 b3293c92e35fcbd1151d516384fce31b108d18fd	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	2
	Information Disclosure Statement (IDS) Form (SB08)	3	3

Warnings:

Information:

4		NPLs.PDF	927656 32c01043df95b81da061a735153dd1bd4acaf881	yes	18

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Non Patent Literature	1	9
Non Patent Literature	10	18

Warnings:

Information:

5	Fee Worksheet (SB06)	fee-info.pdf	32487 1806afd9be48bee01dee9a5387b674018563ca62	no	2

Warnings:

Information:

Total Files Size (in bytes):	10240859

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/256,914	Filing Date 09/06/2016	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT	05/30/2018	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 23	Minus	** 23	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $ 0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $ 0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	marsha R richards
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Docket No.: 074017-0013-01-US-541474
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: H. A. Robinson

TRANSMITTAL FOR DECLARATION FOR PATENT APPLICATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicant submits herewith the executed Declarations (37 CFR 1.63) For Utility or Design Application Using an Application Data Sheet (37 CFR 1.76) in the above-captioned application.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 50-0573, under Order No. 074017-0013-01-US-541474 from which the undersigned is authorized to draw.

Dated: July 13, 2018

Respectfully submitted,

Customer Number: 123223

By _____
Bronwen M. Loeb, Ph.D.
Registration No.: 43,516
DRINKER BIDDLE & REATH LLP
222 Delaware Avenue, Ste 1410
Wilmington, Delaware 19801
202.230.5438 (Phone)
302.351.6938 (Fax)
Attorneys/Agents For Applicant

57692 V502

PTO/AIA/01 (06-12)
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

As the below named inventor, I hereby declare that:

This declaration is directed to:
- [x] The attached application, or
- [ ] United States application or PCT international application number 15/256,914 filed on September 6, 2016.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Jörg BAUER Date (Optional):

Signature: /Bauer/

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

S7672USC2

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

As the below named inventor, I hereby declare that:

This declaration is directed to:
- [x] The attached application, or
- [ ] United States application or PCT international application number 15/256,914 filed on September 6, 2016.

The above-identified application was made or authorized to be made by me.

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LEGAL NAME OF INVENTOR

Inventor: Martin TRUKSA Date (Optional): May 26, 2017

Signature: Martin Truksa

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

5767 2US02

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

As the below named inventor, I hereby declare that:

This declaration is directed to:
- [x] The attached application, or
- [ ] United States application or PCT international application number 15/256,914 filed on September 6, 2016.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Bifang CHENG Date (Optional): May 15, 2017

Signature: Bifang Cheng

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

5763-2U002

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

As the below named inventor, I hereby declare that:

This declaration is directed to:
- [x] The attached application, or
- [ ] United States application or PCT international application number 15/258,914 filed on September 6, 2016.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Guohai WU Date (Optional): April 17, 2017

Signature: Guohai Wu

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

S76724S02

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

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- [ ] United States application or PCT international application number 15/256,914 filed on September 6, 2016

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LEGAL NAME OF INVENTOR

Inventor: Tom WETJEN Date (Optional): 26 Aug 2016

Signature: Tom Wetjen

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

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LEGAL NAME OF INVENTOR

Inventor: Petra CIRPUS Date (Optional) :

Signature: Petra Cirpus

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

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The above-identified application was made or authorized to be made by me.

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I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Xiao QIU Date (Optional): July 3, 2018

Signature: [signature]

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

#85,083,092

Electronic Acknowledgement Receipt

EFS ID:	33172249
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Bronwen M. Loeb/Felicia Bull
Filer Authorized By:	Bronwen M. Loeb
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	13-JUL-2018
Filing Date:	06-SEP-2016
Time Stamp:	13:19:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	TransmittalDeclarations.pdf	37399 9314c5817c8e6f90a74ce47e09c59bf8d1803abd	no	1

Warnings:

Information:					
2	Oath or Declaration filed	Declarations.pdf	5301376 81b5d14255fb4de934f4abfd3c8721bee27720fa	no	7
Warnings:					
Information:					
		Total Files Size (in bytes):	5338775		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 09/19/2018

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
09/19/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

IPDocketWM@dbr.com
penelope.mongelluzzo@dbr.com
DBRIPDocket@dbr.com

Office Action Summary	Application No. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *5/30/18*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☒ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-23* is/are pending in the application.

5a) Of the above claim(s) *6, 11-12, 15 and 17-22* is/are withdrawn from consideration.

6)☒ Claim(s) *1-5,7-10,13,14,16 and 23* is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *9/11/18*.

4) ☐ Other: _____.

DETAILED ACTION

Application Status

1. The present application is being examined under the pre-AIA first to invent provisions.

2. Applicant's amendment filed on May 30, 2018, has been entered.

Claim Disposition

3. Claims 1-23 are pending. Claims 1-5, 7-10, 13-14, 16 and 23 are under examination. Claims 6, 11-12, 15 and 17-22 are withdrawn as directed to a non-elected invention.

4. This application is in condition for allowance except for the following formal matters:

5. Applicant has not canceled the non-elected subject matter with regard to claims 6, 11-12, 15 and 17-22 as these claims are not subject to a rejoinder since not in proper scope based on *In re Ochai*.

6. Prosecution on the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213. A shortened statutory period for reply to this action is set to expire **TWO MONTHS** from the mailing date of this letter.

Conclusion

7. Claims 1-5, 7-10, 13-14, 16 and 23 are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday from 9:00 a.m. to 5:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached at (408) 918-7584. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Hope A. Robinson/

Primary Examiner, Art Unit 1652

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	15/256,914	CIRPUS ET AL.	
	Examiner	Art Unit	
	HOPE ROBINSON	1652	

All participants (applicant, applicant's representative, PTO personnel):

(1) *HOPE ROBINSON*. (3)_____.

(2) *Bronwen Loeb*. (4)_____.

Date of Interview: *11 September 2018*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *6, 11-12, 15 and 17-22*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Contacted applicant's representative to reduce the remaining issues and left a voice message. In the interest of time the attached communication is being mailed and applicant's representative is urged to contact the examiner to expedite further processing of the application.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/HOPE ROBINSON/ Primary Examiner, Art Unit 1652	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

CPC- SEARCHED		
Symbol	**Date**	**Examiner**

CPC COMBINATION SETS - SEARCHED		
Symbol	**Date**	**Examiner**

US CLASSIFICATION SEARCHED			
Class	**Subclass**	**Date**	**Examiner**
NONE		9/11/18	HAR

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

SEARCH NOTES		
Search Notes	**Date**	**Examiner**
Palm Expo inventor names searched	9/11/18	HAR
NPL search (DHA, transgeic Brassica plant, oils, lipids and fatty acids, LCPUFA, triacylglycerides)	9/11/18	HAR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	**US Subclass / CPC Group**	**Date**	**Examiner**

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www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

123223 7590 10/25/2018
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 10/25/2018

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

TITLE OF INVENTION: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0	$0	$1000	01/25/2019

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

123223 7590 10/25/2018
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

TITLE OF INVENTION: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0	$0	$1000	01/25/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
ROBINSON, HOPE A	1652	514-558000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 10/25/2018

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER	
ROBINSON, HOPE A	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 10/25/2018

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *9/19/18*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-16 and 23*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *10/16/18* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

EXAMINER'S AMENDMENT

1. An Examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

2. The present application is being examined under the pre-AIA first to invent provisions.

3. Authorization of this Examiner's amendment was given in a telephone interview with Ms. Bronwen Loeb on October 17, 2018.

4. The Claims have been amended as follows:

Please **cancel** claims 17-22.

EXAMINER'S COMMENTS

5. The Restriction Requirement of record is withdrawn based on the rejoinder herein. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled “Comments on Statement of Reasons for Allowance”.

Conclusion

6. Claims 1-16 and 23 are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi, can be reached at (408) 918-7584. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Hope A. Robinson/

Primary Examiner, Art Unit 1652

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	15/256,914	CIRPUS ET AL.	
	Examiner	Art Unit	
	HOPE ROBINSON	1652	

All participants (applicant, applicant's representative, PTO personnel):

(1) *HOPE ROBINSON*. (3)_____.

(2) *Bronwen Loeb*. (4)_____.

Date of Interview: *16 October 2018*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *6,11,12,15 and 17-22*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Discussed rejoinder and amendments with applicant's representative to place the application in better form. Ms. Loeb authorized the attached examiner's amendment pertaining to the above claims.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/HOPE ROBINSON/ Primary Examiner, Art Unit 1652	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

CPC								
Symbol							**Type**	**Version**
C12N		15	/	8247			F	2013-01-01
C12N		9	/	0083			I	2013-01-01
C12N		9	/	1029			I	2013-01-01
C12N		9	/	0071			I	2013-01-01
C12N		15	/	52			I	2013-01-01
C12P		7	/	6427			I	2013-01-01
C12Y		114	/	19			I	2013-01-01
			/					
			/					
			/					
			/					
			/					
			/					
			/					
			/					

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	17	
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	10/17/18	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
514	558	A	6	1	K	31 / 20 (2006.01.01)						

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
514	2	12	558		

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	17	
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	10/17/18	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

☒ **Claims renumbered in the same order as presented by applicant** ☐ **CPA** ☐ **T.D.** ☐ **R.1.47**

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 17	
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652 (Primary Examiner)	10/17/18 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /H.A.R/

PTO/SB/08b (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	15/256,914 -- Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20150361404	12-17-2015	BASF Plant Science GmbH	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	WAGNER, et al., "Generation of glycerophospholipid molecular species in the yeast Saccharomyces cerevisiae. Fatty acid pattern of phospholipid classes and selective acyl turnover at sn-1 and sn-2 positions", Yeast, Vol. 10, 1994, pp. 1429-1437	
	CB	DIEDRICH, et al., "The natural occurrence of unusual fatty acids. Part 1. Odd numbered fatty acids", Molecular Nutrition & Food Research, Vol. 34, Issue 10, 1990, pp. 935-943	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	10/18/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

91128469.1

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

CPC- SEARCHED		
Symbol	**Date**	**Examiner**

CPC COMBINATION SETS - SEARCHED		
Symbol	**Date**	**Examiner**

US CLASSIFICATION SEARCHED			
Class	**Subclass**	**Date**	**Examiner**
NONE		10/17/18	HAR

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

SEARCH NOTES		
Search Notes	**Date**	**Examiner**
Palm Expo inventor names searched	10/17/18	HAR
NPL search (DHA, transgeic Brassica plant, oils, lipids and fatty acids, LCPUFA, triacylglycerides)	10/17/18	HAR
WEST	10/17/18	HAR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	**US Subclass / CPC Group**	**Date**	**Examiner**
WEST PG PUB		10/17/18	HAR

WEST Search History for Application 15256914

Creation Date: 2018101722:43

Interference Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
(cirpus.in. and qiu.in.) and ((oils, SPACE~lipids, SPACE~fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
Bauer.in.	PGPB, USPT	11179	OR	YES		06-21-2017
(Bauer.in.) and (cirpus.in. and qiu.in. and (oils, SPACE~lipids, SPACE~fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
bauer.in.	PGPB, USPT	11945	OR	YES		10-17-2018
Wu.in.	PGPB, USPT	94767	OR	YES		10-17-2018
cirpus.in.	PGPB, USPT	61	OR	YES		10-17-2018
(cirpus.in.) and (Wu.in.)	PGPB, USPT	8	OR	YES		10-17-2018
(cirpus.in. and Wu.in.) and (bauer.in.)	PGPB, USPT	8	OR	YES		10-17-2018

Prior Art Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
9458436.pn.	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (oils)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (brassica plant)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (4 percent DHA)	USPT	1	OR	YES		06-21-2017

(9458436.pn.) and (sn-1)	USPT	0	OR	YES		06-21-2017
(9458436.pn.) and (sn-2)	USPT	0	OR	YES		06-21-2017
(9458436.pn.) and (sn-2) position	USPT	3490632	OR	YES		06-21-2017
(9458436.pn.) and (sn-2 position)	USPT	1	OR	YES		06-21-2017
(oils, lipids, fatty acids and transgenic Brassica plant)	PGPB, USPT	2231550	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant)) and (DHA)	PGPB, USPT	16649	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA)) and (polyunsaturated fatty acids)	PGPB, USPT	16539	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids)) and (sn-1 or sn-2 or sn-3 position)	PGPB, USPT	8846	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position)) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.)	PGPB, USPT	1564	OR	YES		06-21-2017
qiu.in.	PGPB, USPT	5533	OR	YES		06-21-2017
cirpus.in.	PGPB, USPT	60	OR	YES		06-21-2017
(cirpus.in.) and (qiu.in.)	PGPB, USPT	12	OR	YES		06-21-2017
(cirpus.in. and qiu.in.) and ((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
20160369290	PGPB, USPT	1	OR	YES		06-21-2017
38137303.FMID.	USPT, FPRS, PGPB	9	OR	YES		06-21-2017
(oils or lipids and brassica transgenic plant)	PGPB, USPT	2238756	OR	YES		10-17-2018

((oils or lipids and brassica transgenic plant)) and (fatty acid)	PGPB, USPT	2238756	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant)) and "fatty acid"	PGPB, USPT	443778	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid") and (docosapentaenoic acid)	PGPB, USPT	443778	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid)) and "brassica"	PGPB, USPT	24375	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica") and ((A61K47/44 | C12N15/52 | C12N15/70).CPC.)	PGPB, USPT	404	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica") and (EPA and DHA)	PGPB, USPT	1071	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica" and (EPA and DHA)) and "triacylglycerides"	PGPB, USPT	375	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica" and (EPA and DHA) and "triacylglycerides") and ((C12N9/00 | C12N5/14 | C12P7/6427 | C12P7/64 | C12P7/6409).CPC.)	PGPB, USPT	191	OR	YES		10-17-2018
20160369290	PGPB, USPT	1	OR	YES		10-17-2018
38137303.FMID.	USPT, FPRS, PGPB	11	OR	YES		10-17-2018
(Oils or lipids or fatty acid) and (Brassica)	PGPB, USPT	35573	OR	YES		10-17-2018
((Oils or lipids or fatty acid) and (Brassica)) and (DPA or EPA or DHA)	PGPB, USPT	3280	OR	YES		10-17-2018
cirpus.in.	PGPB, USPT	61	OR	YES		10-17-2018
bauer.in.	PGPB, USPT	11945	OR	YES		10-17-2018
Wu.in.	PGPB, USPT	94767	OR	YES		10-17-2018

PTO/SB/30 (11-17)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	15/256,914
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	H. A. Robinson
Attorney Docket Number	074017-0013-01-US-541474

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).
   - a. [ ] Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
     - i. [ ] Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
     - ii. [ ] Other _____
   - b. [X] Enclosed
     - i. [ ] Amendment/Reply
     - ii. [ ] Affidavit(s)/ Declaration(s)
     - iii. [X] Information Disclosure Statement (IDS)
     - iv. [X] Other Cited References (2)
2. Miscellaneous
   - a. [ ] Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)
   - b. [ ] Other _____
3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   - a. [X] The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments, to Deposit Account No. 50-0573 .
     - i. [X] RCE fee required under 37 CFR 1.17(e)
     - ii. [ ] Extension of time fee (37 CFR 1.136 and 1.17)
     - iii. [ ] Other _____
   - b. [ ] Check in the amount of $ _____ enclosed
   - c. [ ] Payment by credit card (Form PTO-2038 enclosed)
   - d. [X] Payment by EFS-Web

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature		Date	October 30, 2018
Name (Print/Type)	Bronwen M. Loeb, Ph.D.	Registration No.	43,516

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being EFS-Web transmitted to the United States Patent and Trademark Office (USPTO), deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the USPTO on the date shown below.

Signature			
Name (Print/Type)		Date	

PTO/SB/08a/08b (07-09)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	15/256,914
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	H. A. Robinson
Attorney Docket Number	074017-0013-01-US-541474

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	A	GUNSTONE FD, "Movements towards tailor-made fats", Progress in Lipid Research, Vol. 37, Issue 5, November 1998, pp. 277-305.	
	B	THELEN, et al., "Metabolic Engineering of Fatty Acid Biosynthesis in Plants", Metabolic Engineering, Vol. 4, Issue 1, 2002, pp. 12-21.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS | Examiner: H. A. Robinson

INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

[x] **Under 37 C.F.R. § 1.97(b):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(b), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. To the undersigned's knowledge, this IDS is being filed before the mailing date of a first Office Action on the merits, before the mailing date of a first Office Action on the merits after filing an RCE under § 1.114, or within three months of the application filing date.

[ ] **Under 37 C.F.R. § 1.97(c):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(c), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. This IDS is being filed after the events recited in § 1.97(b) but, to the undersigned's knowledge, before the mailing date of a Final Office Action, a Notice of Allowance, or another action that closes prosecution in the application.

[ ] The fee of $240.00 set forth in § 1.17(p) is included herein;

[ ] Applicant submits that each item of information contained in this IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this IDS. 37 C.F.R. § 1.97(e)(1).

☐ Applicant submits that no item of information contained in this IDS was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in this IDS was known to any individual designated in § 1.56(c) more than three months prior to the filing of this IDS.
37 C.F.R. § 1.97(e)(2).

☐ **Under 37 C.F.R. § 1.97(d):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(d), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. This IDS is being filed after the events recited in § 1.97(c) but before payment of the issue fee.

☐ The fee of $240.00 set forth in § 1.17(p) is included herein; and

☐ Applicant submits that each item of information contained in this IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this IDS.

☐ Applicant submits that no item of information contained in this IDS was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in this IDS was known to any individual designated in § 1.56(c) more than three months prior to the filing of this IDS.
37 C.F.R. § 1.97(e)(2).

☐ **Under 37 C.F.R. § 1.97(i):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(i), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. This IDS is being filed after the events recited in § 1.97(d). Applicant requests that the IDS be placed in the file.

☐ A search report or other listing of documents from a counterpart, related, or other application dated and having documents cited thereon is attached for the Examiner's consideration. Any of these documents not previously cited, and any additional documents are listed on the PTO/SB/08 Form.

☒ Applicant respectfully requests that the Examiner consider the listed documents

and evidence that consideration by making appropriate notations on the attached form. As for any document listed on the accompanying PTO/SB/08 Form that is in a language other than English, relevance can be understood from an enclosed English abstract or corresponding English-language document or at least partial translation or from mention in the specification or in a search report for a corresponding application.

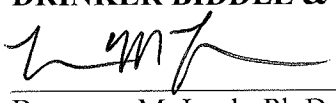
This submission does not represent that a search has been made or that no better art exists and does not constitute an admission that any of the listed documents are material or constitute "prior art." If it should be determined that any of the listed documents do not constitute "prior art" under United States law, Applicant reserves the right to present to the Office the relevant facts and law regarding the appropriate status of such documents.

Applicant further reserves the right to take appropriate action to establish the patentability of the disclosed invention over the listed documents, should any of the documents be applied against the claims of the present application.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this Application, including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required and including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Respectfully submitted,

DRINKER BIDDLE & REATH LLP



Bronwen M. Loeb, Ph.D.
Registration No.: 43,516
DATED: October 30, 2018

CUSTOMER NO. 123223

DRINKER BIDDLE & REATH LLP
222 Delaware Avenue, Ste 1410
Wilmington, Delaware 19801
Tel: 302.467.4200
Fax: 302.351.6938

Electronic Patent Application Fee Transmittal

Application Number:	15256914
Filing Date:	06-Sep-2016
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Filer:	Bronwen M. Loeb/Felicia Bull
Attorney Docket Number:	074017-0013-01-US

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 1ST REQUEST	1801	1	1300	1300
		Total in USD ($)		**1300**

Electronic Acknowledgement Receipt

EFS ID:	34163003
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Bronwen M. Loeb/Felicia Bull
Filer Authorized By:	Bronwen M. Loeb
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	30-OCT-2018
Filing Date:	06-SEP-2016
Time Stamp:	16:34:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1300
RAM confirmation Number	103118INTEFSW16361500
Deposit Account	500573
Authorized User	Felicia Bull

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	RCE.pdf	72675 17ce7283eeffc987e987c02b13ca50160d89443e	no	1
Warnings:					
This is not a USPTO supplied RCE SB30 form.					
Information:					
2		IDS.pdf	215533 e8ef024613081591ab4fdd1b07f848460d6e7d68	yes	4

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Information Disclosure Statement (IDS) Form (SB08)	4	4
	Transmittal Letter	1	3

Warnings:					
Information:					
3	Non Patent Literature	074017-0013-01-US-541474_-_Gunstone_1998_Progress_in_Lipid_Research.pdf	263824 c942f871155f6a90c0a22c9e0b279360b186102f	no	29
Warnings:					
Information:					
4	Non Patent Literature	074017-0013-01-US-541474_-_Thelen_2002_Metabolic_Engineering.pdf	230560 0b5b60745502d58ddaf72909f7f334c5e0b5ad65	no	10
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30602 5c74dd418e475090cb45ef8ae98a008cb5729605	no	2
Warnings:					

Information:	
Total Files Size (in bytes):	813194

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/256,914	09/06/2016	1652	1840	074017-0013-01-US	22	2

CONFIRMATION NO. 4050

CORRECTED FILING RECEIPT

123223
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

OC000000103941010

Date Mailed: 11/21/2018

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Petra Cirpus, Mannheim, GERMANY;
Jörg Bauer, Limburgerhof, GERMANY;
Xiao Qiu, Saskatoon, CANADA;
Guohai Wu, Saskatoon, CANADA;
Bifang Cheng, Saskatoon, CANADA;
Martin Truksa, Saskatoon, CANADA;
Tom Wetjen, Mannheim, GERMANY;

Applicant(s)

BASF Plant Science GmbH, Ludwigshafen, GERMANY;

Power of Attorney: The patent practitioners associated with Customer Number 123223

Domestic Priority data as claimed by applicant

This application is a CON of 12/280,090 08/20/2008
which is a 371 of PCT/EP2007/051675 02/21/2007

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 06120309.7 09/07/2006 No Access Code Provided
GERMANY 102006008030.0 02/21/2006

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 09/13/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/256,914**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

OILS, LIPIDS AND PATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Docket No.: 074017-0013-01-US-541474
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT | Examiner: H. A. Robinson

REQUEST FOR CORRECTED FILING RECEIPT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Attached is a marked-up copy of the Corrected Filing Receipt mailed November 21, 2018 by the PTO in the above application and for which issuance of a corrected filing receipt is respectfully requested.

There is an error with respect to the following data which is:

[X] incorrectly entered [...] Spelling Error ("Fatty" misspelled as "Patty")

Error In	**Correct Data**
[X] Title	OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT

Applicant additionally requests that all pertinent U.S. Patent and Trademark Office records relating to the subject application be changed to reflect this correction.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this

application including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573, under Order No. 074017-0013-01-US-541474, from which the undersigned is authorized to draw.

Dated: November 30, 2018

Respectfully submitted,

By _____

Customer Number: 123223

Bronwen M. Loeb, Ph.D.
Registration No.: 43,516
DRINKER BIDDLE & REATH LLP
1500 K Street., N.W.
Washington, DC 20005
202.230.5438 (Phone)
302.351.6938 (Fax)
Attorneys/Agents For Applicant

MARKED UP

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/256,914	09/06/2016	1652	1840	074017-0013-01-US	22	2

CONFIRMATION NO. 4050

CORRECTED FILING RECEIPT

123223
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

OC000000103941010

Date Mailed: 11/21/2018

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

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Power of Attorney: The patent practitioners associated with Customer Number 123223

Domestic Priority data as claimed by applicant

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which is a 371 of PCT/EP2007/051675 02/21/2007

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EUROPEAN PATENT OFFICE (EPO) 06120309.7 09/07/2006 No Access Code Provided
GERMANY 102006008030.0 02/21/2006

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 09/13/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/256,914**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT

~~OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT~~

Preliminary Class

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

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SelectUSA

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community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Electronic Acknowledgement Receipt

EFS ID:	34452349
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	OILS, LIPIDS AND PATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Bronwen M. Loeb/Felicia Bull
Filer Authorized By:	Bronwen M. Loeb
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	30-NOV-2018
Filing Date:	06-SEP-2016
Time Stamp:	15:35:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		SupplementalAmendment.pdf	377848 b2556fa289d330b1b38a94d886470a896704ca20	yes	10

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Supplemental Response or Supplemental Amendment	1	1
	Claims	2	7
	Applicant Arguments/Remarks Made in an Amendment	8	10

Warnings:					
Information:					
2	Request for Corrected Filing Receipt	RequestCorrectedOFR.pdf	298419 f8ca3a18eb5834a1807ba1ff66eb0211a68d8ee3	no	6
Warnings:					
Information:					
		Total Files Size (in bytes):	676267		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 074017-0013-01-US-541474
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Petra CIRPUS et al.

Application No.: 15/256,914 | Confirmation No.: 4050

Filed: September 6, 2016 | Art Unit: 1652

For: OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT | Examiner: H. A. Robinson

APPLICANT'S RECORD OF SUBSTANCE OF EXAMINER-INITIATED INTERVIEW

&

SUPPLEMENTAL AMENDMENT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Amendment is being filed pursuant to an Examiner-initiated Interview subsequent to the Request for Continued Examiner with an Information Disclosure Statement filed October 30, 2018. Please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper; and

Remarks/Arguments begin on page 8 of this paper.

AMENDMENTS TO THE CLAIMS

Please amend the claims of the above-identified application as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended): Oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant, wherein said polyunsaturated fatty acids comprise at least 20% by weight of eicosapentaenoic acid (EPA), at least 2% by weight of docosapentaenoic acid (DPA), and at least 4% by weight of docosahexaenoic acid (DHA) based on the total fatty acids in the transgenic plant in the form of triacylglycerides.

2. (Previously Presented): The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at least 24% by weight of EPA is present in the sn-1, sn-2 or sn-3 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 1.5% by weight of DPA is present in the sn-1, sn-2 or sn-3 position; and

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-1, sn-2 or sn-3 position.

3. (Previously Presented): The oils, lipids and/or fatty acids of claim 1, wherein:

a) said polyunsaturated fatty acids comprise at least 20% by weight of EPA and at

least 24% by weight of EPA is present in the sn-2 position;

b) said polyunsaturated fatty acids comprise at least 2% by weight of DPA and at least 3% by weight of DPA is present in the sn-2 position; and

c) said polyunsaturated fatty acids comprise at least 4% by weight of DHA and at least 3% by weight of DHA is present in the sn-2 position.

4. (Currently Amended): The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise:

a) at least 20% by weight of EPA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in the form of triacylglycerides;

b) at least 20% by weight of EPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in the form of triacylglycerides; or

c) at least 2% by weight of DPA and at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in the form of triacylglycerides.

5. (Original): The oils, lipids and/or fatty acids of claim 1, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

6. (Currently Amended): The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in the form of

triacylglycerides based on the total fatty acids in the transgenic plant.

7. (Currently Amended): The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in the form of triacylglycerides based on the total fatty acids in the transgenic plant.

8. (Original) The oils, lipids and/or fatty acids of claim 1, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

9. (Previously Presented): Oils, lipids and/or fatty acids produced by a transgenic *Brassica* plant, wherein said oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω3-fatty acids based on the total fatty acids in the transgenic plant.

10. (Original) The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise 60 to 85% by weight of polyunsaturated fatty acids based on the total fatty acids in the transgenic plant.

11. (Currently Amended): The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 20% by weight of EPA based on the total fatty acids in the transgenic plant in the form of triacylglycerides.

12. (Currently Amended): The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 2% by weight of DPA based on the total fatty acids in the transgenic plant in the form of triacylglycerides.

13. (Currently Amended) The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 4% by weight of DHA based on the total fatty acids in the transgenic plant in the form of triacylglycerides.

14. (Original): The oils, lipids and/or fatty acids of claim 9, wherein said polyunsaturated fatty acids comprise at least 30% long chain polyunsaturated fatty acids (LCPUFAs) based on the total fatty acids in the transgenic plant, and wherein the LCPUFAs are C20 and/or C22 fatty acid molecules having at least four double bonds.

15. (Currently Amended): The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise about 25% by weight of EPA in the form of triacylglycerides based on the total fatty acids in the transgenic plant.

16. (Currently Amended): The oils, lipids and/or fatty acids of claim 9, wherein said oils, lipids and/or fatty acids comprise a total amount of about 30% by weight of EPA and DHA in the form of triacylglycerides based on the total fatty acids in the transgenic plant.

17-22. (Cancelled).

23. (Previously presented): The oils, lipids and/or fatty acids of claim 1, comprising:

a) at least 4% by weight of DHA and at least 2% by weight of DPA based on the total fatty acids in the transgenic plant;

b) at least 4% by weight of DHA and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant; or

c) at least 4% by weight of DHA, at least 2% by weight of DPA, and at least 20% by weight of EPA based on the total fatty acids in the transgenic plant.

24. (New): A method for producing oils, lipids and/or fatty acids of claim 1, comprising expressing in a *Brassica* plant a nucleic acid encoding a Δ6-desaturase, a nucleic acid encoding a Δ5-desaturase, a nucleic acid encoding a Δ6-elongase, a nucleic acid encoding a ω3-desaturase, a nucleic acid encoding a Δ5-elongase, and a nucleic acid encoding a Δ4-desaturase, wherein said nucleic acid encoding a Δ5-elongase is codon-optimized by adapting to the codon usage of *Brassica*.

25. (New): The method of claim 24, wherein said nucleic acid encoding a Δ5-elongase comprises a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence of SEQ ID NO: 64, and wherein said nucleotide sequence is obtained by adapting at least 30% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of *Brassica*.

26. (New): The method of claim 25, wherein said nucleotide sequence has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the nucleotide sequence of SEQ ID NO: 64, or wherein said nucleotide sequence encodes a polypeptide having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65.

27. (New): The method of claim 25, wherein said nucleotide sequence is adapted based on the natural frequency of individual codons.

28. (New): The method of claim 25, wherein said nucleotide sequence is obtained by adapting at least 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of *Brassica*.

29. (New): The method of claim 24, wherein said nucleic acid encoding a Δ5-elongase comprises a translated section coding for a protein having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65, and wherein the translated section comprises a nucleotide sequence obtained by adapting at least 30% or 40% of the nucleotide sequence of SEQ ID NO: 109 to the codon usage of *Brassica*.

REMARKS

Entry of the foregoing amendments and reconsideration of the subject application are respectfully requested in light of the comments which follow.

1. Status of the Claims

Claims 1-16 and 23 are pending, and claims 17-22 are cancelled. Claims 1, 4, 6, 7, 11-13, 15, and 16 are amended.

New claims 24-29 are presented, and are supported throughout the specification, for instance, by original claims 17-22; at page 11, lines 12-16; and at page 58, lines 7-14 (referring to PCT specification).

No prohibited new matter is believed to be introduced by the amended claim set.

Claims 1-16 and 23-29 are pending.

2. Statement of Substance of an Examiner-Initiated Interview

Examiner Hope Robinson contacted Applicants' undersigned representative on November 27, 2018 requesting a telephonic interview to discuss possible amendments to put the application in condition for allowance. The interview was held November 27, 2018.

During the Examiner-initiated interview, the Examiner stated that the application was close to allowance. However, the Examiner indicated that claim clarity could be improved by reciting "in the form of triglycerides" in claims 1, 4, 6, 7, 11-13, 15, and 16.

The Examiner also indicated that she was willing to consider rejoining withdrawn claims 17-22 to the elected subject matter examined in this application. However, she indicated that

claims amendments would be needed to improve claim form and clarity. Specifically, the Examiner requested that in claim 18 the % identity with regard to SEQ ID NO: 64 be amended to 90%, consistent with the allowed parent application. The Examiner also indicated that claim 19, which depends from claim 18, would thenneed to be amended so that the lowest % identity is 90%. The Examiner also suggested that claim 20 does not further limit claim 18, alleging that adapting codon usage necessarily involves taking into account natural frequency.

Examiner Robinson is thanked for the courtesies extended to Applicants' undersigned representative during the November 27, 2018 telephonic interviews. This filing is made pursuant to and consistent with the Examiner-initiated telephonic interview held November 27, 2018.

3. Comments regarding Interview

This amendment is filed pursuant to the Examiner-initiated Telephonic Interview and the Examiner's suggested claim amendments. The amendments to claims 1, 4, 6, 7, 11-13, 15, and 16, and new claims 25 and 26 (corresponding to cancelled claims 18 and 19 respectively) implement the Examiner's suggestions.

Regarding claim 27 (corresponding to cancelled claim 20), Applicant notes that the specification discloses two possible frequency considerations for adapting codon usage: (1) adapting codon usage to the frequency in the target organism, and (2) adapting based on the natural frequency of the codon. *See* page 11, lines 12-16 (PCT specification). Therefore, to improve the clarity, claim 27 recites "adapted based on the natural frequency of individual codons. Applicant submits that claim 27 further limits claim 25, obviating the Examiner's concern regarding this claim.

CONCLUSIONS

In view of the foregoing, Applicant(s) submit(s) that the pending claims are in condition for allowance, and respectfully request(s) reconsideration and timely allowance of the pending claims. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact Applicants' undersigned representative to expedite prosecution. A favorable action is awaited.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573, from which the undersigned is authorized to draw.

Dated: November 30, 2018

Respectfully submitted,

By _____

Customer Number: 123223

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1500 K Street., N.W.
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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 12/05/2018

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
12/05/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DBRIPDocket@dbr.com
IPDocketWM@dbr.com

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	15/256,914	CIRPUS ET AL.	
	Examiner	Art Unit	
	HOPE ROBINSON	1652	

All participants (applicant, applicant's representative, PTO personnel):

(1) *HOPE ROBINSON*. (3)_____.

(2) *Bronwen Loeb*. (4)_____.

Date of Interview: *27 November 2018*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1-23*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The remaining issues were discussed with applicant's representative with respect to language clarification concerning the above claims and rejoinder. Ms. Loeb requested time to consult with the applicants and responded on 11/29/18 that she will file a supplemental amendment.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/HOPE ROBINSON/ Primary Examiner, Art Unit 1652	

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/256,914	09/06/2016	1652	1840	074017-0013-01-US	22	2

CONFIRMATION NO. 4050

CORRECTED FILING RECEIPT

123223
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

OC000000104225389

Date Mailed: 12/06/2018

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

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Applicant(s)

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Power of Attorney: The patent practitioners associated with Customer Number 123223

Domestic Priority data as claimed by applicant

This application is a CON of 12/280,090 08/20/2008
which is a 371 of PCT/EP2007/051675 02/21/2007

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 06120309.7 09/07/2006 No Access Code Provided
GERMANY 102006008030.0 02/21/2006

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If Required, Foreign Filing License Granted: 09/13/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/256,914**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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NOTICE OF ALLOWANCE AND FEE(S) DUE

123223 7590 12/19/2018
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 12/19/2018

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

TITLE OF INVENTION: OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0	$0	$1000	03/19/2019

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

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I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: **(571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

123223 7590 12/19/2018
Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date indicated below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

TITLE OF INVENTION: OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0	$0	$1000	03/19/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
ROBINSON, HOPE A	1652	514-558000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required) ❑ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 12/19/2018

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER	
ROBINSON, HOPE A	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 12/19/2018

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 1652	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *11/30/18*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-16 and 23-29*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/HOPE ROBINSON/
Primary Examiner, Art Unit 1652

EXAMINER'S COMMENTS

1. The present application is being examined under the pre-AIA first to invent provisions.

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on October 30, 2018, has been entered.

3. The Restriction Requirement of record is withdrawn based on the rejoinder of non-elected claims. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays,

should preferably accompany the issue fee. Such submissions should be clearly labeled “Comments on Statement of Reasons for Allowance”.

Conclusion

4. Claims 1-16 and 23-29 are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday. If attempts to reach the examiner by telephone are unsuccessful, the examiner’s supervisor, Robert Mondesi, can be reached at (408) 918-7584. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Hope A. Robinson/

Primary Examiner, Art Unit 1652

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

CPC							
Symbol						**Type**	**Version**
C12N		15	/	8247		F	2013-01-01
C12N		9	/	0083		I	2013-01-01
C12N		9	/	1029		I	2013-01-01
C12N		9	/	0071		I	2013-01-01
C12N		15	/	52		I	2013-01-01
C12P		7	/	6427		I	2013-01-01
C12Y		114	/	19		I	2013-01-01
			/				
			/				
			/				
			/				
			/				
			/				
			/				
			/				

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	23	
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	12/7/18	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
514	558	A	6	1	K	31 / 20 (2006.01.01)						

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
514	2	12	558		

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	23	
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	12/7/18	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	**Art Unit**
	HOPE ROBINSON	1652

☒ **Claims renumbered in the same order as presented by applicant** ☐ **CPA** ☐ **T.D.** ☐ **R.1.47**

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 23	
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652 (Primary Examiner)	12/7/18 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

PTO/SB/08a/08b (07-09)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /H.A.R./

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	15/256,914
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	H. A. Robinson
Attorney Docket Number	074017-0013-01-US-541474

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	A	GUNSTONE FD, "Movements towards tailor-made fats", Progress in Lipid Research, Vol. 37, Issue 5, November 1998, pp. 277-305.	
	B	THELEN, et al., "Metabolic Engineering of Fatty Acid Biosynthesis in Plants", Metabolic Engineering, Vol. 4, Issue 1, 2002, pp. 12-21.	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	11/26/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15256914	CIRPUS ET AL.
	Examiner	Art Unit
	HOPE ROBINSON	1652

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
NONE		12/7/18	HAR

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

SEARCH NOTES		
Search Notes	Date	Examiner
Palm Expo inventor names searched	10/17/18	HAR
NPL search (DHA, transgeic Brassica plant, oils, lipids and fatty acids, LCPUFA, triacylglycerides)	10/17/18	HAR
WEST	10/17/18	HAR
WEST	12/7/18	HAR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
WEST PG PUB		10/17/18	HAR

WEST Search History for Application 15256914

Creation Date: 2018120720:01

Interference Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
(cirpus.in. and qiu.in.) and ((oils, SPACE~lipids, SPACE~fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
Bauer.in.	PGPB, USPT	11179	OR	YES		06-21-2017
(Bauer.in.) and (cirpus.in. and qiu.in. and (oils, SPACE~lipids, SPACE~fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
bauer.in.	PGPB, USPT	11945	OR	YES		10-17-2018
Wu.in.	PGPB, USPT	94767	OR	YES		10-17-2018
cirpus.in.	PGPB, USPT	61	OR	YES		10-17-2018
(cirpus.in.) and (Wu.in.)	PGPB, USPT	8	OR	YES		10-17-2018
(cirpus.in. and Wu.in.) and (bauer.in.)	PGPB, USPT	8	OR	YES		10-17-2018

Prior Art Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
9458436.pn.	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (oils)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (brassica plant)	USPT	1	OR	YES		06-21-2017
(9458436.pn.) and (4 percent DHA)	USPT	1	OR	YES		06-21-2017

(9458436.pn.) and (sn-1)	USPT	0	OR	YES		06-21-2017
(9458436.pn.) and (sn-2)	USPT	0	OR	YES		06-21-2017
(9458436.pn.) and (sn-2) position	USPT	3490632	OR	YES		06-21-2017
(9458436.pn.) and (sn-2 position)	USPT	1	OR	YES		06-21-2017
(oils, lipids, fatty acids and transgenic Brassica plant)	PGPB, USPT	2231550	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant)) and (DHA)	PGPB, USPT	16649	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA)) and (polyunsaturated fatty acids)	PGPB, USPT	16539	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids)) and (sn-1 or sn-2 or sn-3 position)	PGPB, USPT	8846	OR	YES		06-21-2017
((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position)) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.)	PGPB, USPT	1564	OR	YES		06-21-2017
qiu.in.	PGPB, USPT	5533	OR	YES		06-21-2017
cirpus.in.	PGPB, USPT	60	OR	YES		06-21-2017
(cirpus.in.) and (qiu.in.)	PGPB, USPT	12	OR	YES		06-21-2017
(cirpus.in. and qiu.in.) and ((oils, lipids, fatty acids and transgenic Brassica plant) and (DHA) and (polyunsaturated fatty acids) and (sn-1 or sn-2 or sn-3 position) and ((A23V2002/00 | A23L33/12 | A23L33/115 | A23K20/158).CPC.))	PGPB, USPT	5	OR	YES		06-21-2017
20160369290	PGPB, USPT	1	OR	YES		06-21-2017
38137303.FMID.	USPT, FPRS, PGPB	9	OR	YES		06-21-2017
(oils or lipids and brassica transgenic plant)	PGPB, USPT	2238756	OR	YES		10-17-2018

((oils or lipids and brassica transgenic plant)) and (fatty acid)	PGPB, USPT	2238756	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant)) and "fatty acid"	PGPB, USPT	443778	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid") and (docosapentaenoic acid)	PGPB, USPT	443778	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid)) and "brassica"	PGPB, USPT	24375	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica") and ((A61K47/44 | C12N15/52 | C12N15/70).CPC.)	PGPB, USPT	404	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica") and (EPA and DHA)	PGPB, USPT	1071	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica" and (EPA and DHA)) and "triacylglycerides"	PGPB, USPT	375	OR	YES		10-17-2018
((oils or lipids and brassica transgenic plant) and "fatty acid" and (docosapentaenoic acid) and "brassica" and (EPA and DHA) and "triacylglycerides") and ((C12N9/00 | C12N5/14 | C12P7/6427 | C12P7/64 | C12P7/6409).CPC.)	PGPB, USPT	191	OR	YES		10-17-2018
20160369290	PGPB, USPT	1	OR	YES		10-17-2018
38137303.FMID.	USPT, FPRS, PGPB	11	OR	YES		10-17-2018
(Oils or lipids or fatty acid) and (Brassica)	PGPB, USPT	35573	OR	YES		10-17-2018
((Oils or lipids or fatty acid) and (Brassica)) and (DPA or EPA or DHA)	PGPB, USPT	3280	OR	YES		10-17-2018
cirpus.in.	PGPB, USPT	61	OR	YES		10-17-2018
bauer.in.	PGPB, USPT	11945	OR	YES		10-17-2018
Wu.in.	PGPB, USPT	94767	OR	YES		10-17-2018

(Wu.in.) and (bauer.in.)	PGPB, USPT	27	OR	YES		10-17-2018
(Wu.in. and bauer.in.) and (cirpus.in.)	PGPB, USPT	8	OR	YES		10-17-2018
((Oils or lipids or fatty acid) and (Brassica) and (DPA or EPA or DHA)) and ((C12N15/82 | Y02A40/146 | C07K14/415 | C12P7/6427 | C12P7/6409 | C12P7/64 | A23K20/158).CPC.)	PGPB, USPT	1138	OR	YES		10-17-2018
((Oils or lipids or fatty acid) and (Brassica) and (DPA or EPA or DHA) and ((C12N15/82 | Y02A40/146 | C07K14/415 | C12P7/6427 | C12P7/6409 | C12P7/64 | A23K20/158).CPC.)) and (Wu.in. and bauer.in. and cirpus.in.)	PGPB, USPT	4	OR	YES		10-17-2018
(Oils, lipids, fatty acid)	PGPB, USPT	n/a	OR	YES		12-07-2018
(oils, lipids and fatty acid) and (transgenic brassica plant)	PGPB, USPT	655171	OR	YES		12-07-2018
((oils, lipids and fatty acid) and (transgenic brassica plant)) and "EPA"	PGPB, USPT	25720	OR	YES		12-07-2018
((oils, lipids and fatty acid) and (transgenic brassica plant) and "EPA") and "DPA"	PGPB, USPT	2444	OR	YES		12-07-2018
((oils, lipids and fatty acid) and (transgenic brassica plant) and "EPA" and "DPA") and "DHA"	PGPB, USPT	2288	OR	YES		12-07-2018
wu.in.	PGPB, USPT	96040	OR	YES		12-07-2018
qiu.in.	PGPB, USPT	6607	OR	YES		12-07-2018
bauer.in.	PGPB, USPT	12040	OR	YES		12-07-2018
cirpus.in.	PGPB, USPT	61	OR	YES		12-07-2018
(cirpus.in.) and (bauer.in.)	PGPB, USPT	27	OR	YES		12-07-2018
(cirpus.in. and bauer.in.) and (qiu.in.)	PGPB, USPT	13	OR	YES		12-07-2018
(cirpus.in. and bauer.in. and qiu.in.) and (wu.in.)	PGPB, USPT	8	OR	YES		12-07-2018
(cirpus.in. and bauer.in. and qiu.in. and wu.in.	PGPB,	6	OR	YES		12-07-2018

) and ((oils, lipids and fatty acid) and (transgenic brassica plant) and "EPA" and "DPA" and "DHA")	USPT					
20160369290	PGPB, USPT	1	OR	YES		12-07-2018
38137303.FMID.	USPT, FPRS, PGPB	11	OR	YES		12-07-2018
38137303.FMID.	USPT, FPRS, PGPB	11	OR	YES		12-07-2018
(cirpus.in. and bauer.in. and qiu.in. and wu.in. and (oils, lipids and fatty acid) and (transgenic brassica plant) and "EPA" and "DPA" and "DHA") and ((C12N15/52 | A23D9/00).CPC.)	PGPB, USPT	6	OR	YES		12-07-2018

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 12/20/2018

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
12/20/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Application No. : 15256914
Applicant : Cirpus
Filing Date : 09/06/2016
Date Mailed : 12/20/2018

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Quang Nguyen/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 15256914

NON-COMPLIANT ILLUSTRATIONS IN SPECIFICATION OR CLAIMS

☐ **The illustration(s) in the location(s) referenced below do not come within the exceptions of 37 CFR 1.58(a). Please delete the illustration(s) from the specification or claims, provided the illustration(s) as part of the formal drawing(s) in accordance with 37 CFR 1.84, and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):**

- ☐ Specification document coded dated , page(s) .
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

☒ **The chemical formula(s), mathematical formula(s), or table(s) in the location(s) referenced below contain shading (color/grayscale) and therefore do not comply with 37 CFR 1.52 and do not come within the exceptions of 37 CFR 1.58(a). Please delete chemical formula(s), mathematical formula(s), or table(s) with shading from the specification or claims, provide them as formal color drawing(s) in accordance with 37 CFR 1.84, including petition, fee, and amendment in accordance with 37 CFR 1.84(a)(2), and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):**

- ☒ Specification document coded SPEC dated 10/23/2017, page(s) 81-86 (Tables 1 - 6).
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

Docket No.: 074017-0013-01-US-541474
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Petra CIRPUS et al.	Allowed: December 19, 2018
Application No.: 15/256,914	Confirmation No.: 4050
Filed: September 6, 2016	Art Unit: 1652
For: OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT	Examiner: H. A. Robinson

AMENDMENT IN RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS AND SUBMISSION OF SUBSTITUTE SPECIFICATION

MS Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Notice to File Corrected Application Papers dated December 20, 2018, a response date to which runs through February 20, 2019, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2 of this paper; and

Remarks/Arguments begin on page 3 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the specification filed October 23, 2017 with the substitute specification that is submitted herewith.

Attachment: Substitute Specification-Clean (88 pages)

REMARKS

In response to the Notice to File Corrected Application Papers dated December 20, 2018, a substitute specification is submitted herewith. No new matter has been added.

In the substitute specification, the shading in Tables 1-6 is removed. See pages 83-88 of the Substitute Specification. This description of the changes is believed sufficient to identify the corrections, there no marked up Substitute Specification showing the changes is provided.

A copy of the Notice to File Corrected Application Papers is attached.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Dated: January 16, 2019

Respectfully submitted,

Customer Number: 123223

By _____

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See attachment(s).

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Application No. 15256914

NON-COMPLIANT ILLUSTRATIONS IN SPECIFICATION OR CLAIMS

☐ The illustration(s) in the location(s) referenced below do not come within the exceptions of 37 CFR 1.58(a). Please delete the illustration(s) from the specification or claims, provided the illustration(s) as part of the formal drawing(s) in accordance with 37 CFR 1.84, and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):

- ☐ Specification document coded dated , page(s) .
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

☒ The chemical formula(s), mathematical formula(s), or table(s) in the location(s) referenced below contain shading (color/grayscale) and therefore do not comply with 37 CFR 1.52 and do not come within the exceptions of 37 CFR 1.58(a). Please delete chemical formula(s), mathematical formula(s), or table(s) with shading from the specification or claims, provide them as formal color drawing(s) in accordance with 37 CFR 1.84, including petition, fee, and amendment in accordance with 37 CFR 1.84(a)(2), and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):

- ☒ Specification document coded SPEC dated 10/23/2017, page(s) 81-86 (Tables 1 - 6).
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

Oils, Lipids And Fatty Acids Produced in Transgenic Brassica Plant

RELATED APPLICATIONS

This application is a continuation of patent application Serial No. 12/280,090 filed August 20, 2008, which is a national stage application (under 35 U.S.C. § 371) of PCT/EP2007/051675, filed February 21, 2007, which claims benefit of German application 10 2006 008 030.0, filed February 21, 2006 and European application 06120309.7, filed September 7, 2006. The entire content of each aforementioned application is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING

The Sequence Listing associated with this application is filed in electronic format *via* EFS-Web and hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is Sequence_Listing_074017_0013_01. The size of the text file is 730 KB, and the text file was created on September 2, 2016.

The present invention relates to a process for the production of eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in transgenic plants, providing in the plant at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity, where the nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified by comparison with the nucleic acid sequence in the organism from which the sequence is derived in that it is adapted to the codon usage in one or more plant species.

In a preferred embodiment there is additionally provision of further nucleic acid sequences which code for a polypeptide having the activity of an ω3-desaturase and/or of a Δ4-desaturase in the plant.

In a further preferred embodiment there is provision of further nucleic acid sequences which code for acyl-CoA dehydrogenase(s), acyl-ACP (acyl carrier protein) desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyl transferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-

coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) in the plant.

The invention furthermore relates to recombinant nucleic acid molecules comprising at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species.

A further part of the invention relates to oils, lipids and/or fatty acids which have been produced by the process according to the invention, and to their use.

Finally, the invention also relates to transgenic plants which have been produced by the process of the invention or which comprise a recombinant nucleic acid molecule of the invention, and to the use thereof as foodstuffs or feedstuffs.

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3-phosphate, and the addition or modification of a polar head group. Usual lipids which are used in membranes comprise phospholipids, glycolipids, sphingolipids and phosphoglycerides. Fatty acid synthesis starts with the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase or into acetyl-ACP by acetyl transacylase. After condensation reaction, these two product molecules together form acetoacetyl-ACP, which is converted via a series of condensation, reduction and dehydration reactions so that a saturated fatty acid molecule with the desired chain length is obtained. The production of the unsaturated fatty acids from these molecules is catalyzed by specific desaturases, either aerobically by means of molecular oxygen or anaerobically (regarding the fatty acid synthesis in microorganisms, see F.C. Neidhardt et al. (1996) E. coli and Salmonella. ASM Press: Washington, D.C., p. 612-636 and references cited therein; Lengeler et al. (Ed.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, and the references therein, and Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 and the references therein). To undergo the further elongation steps, the resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This

is made possibly by acyl-CoA:lysophospholipid acyltransferases. Moreover, these enzymes are capable of transferring the elongated fatty acids from the CoA esters back to the phospholipids. If appropriate, this reaction sequence can be followed repeatedly.

Furthermore, fatty acids must subsequently be transported to various modification sites and incorporated into the triacylglycerol storage lipid. A further important step during lipid synthesis is the transfer of fatty acids to the polar head groups, for example by glycerol fatty acid acyltransferase (see Frentzen, 1998, Lipid, 100(4-5):161-166).

An overview of the biosynthesis of fatty acids in plants, desaturation, the lipid metabolism and the membrane transport of lipidic compounds, beta-oxidation, the modification of fatty acids, cofactors and the storage and assembly of triacylglycerol, including the references is given by the following papers: Kinney (1997) Genetic Engineering, Ed.: JK Setlow, 19:149-166; Ohlrogge and Browse (1995) Plant Cell 7:957-970; Shanklin and Cahoon (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker (1996) Genetic Engeneering, Ed.: JK Setlow, 18:111-13; Gerhardt (1992) Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl (1995) Biochim. Biophys Acta 1256:181-186; Kunau et al. (1995) Prog. Lipid Res. 34:267-342; Stymne et al. (1993) in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Ed.: Murata und Somerville, Rockville, American Society of Plant Physiologists, 150-158; Murphy & Ross (1998) Plant Journal. 13(1):1-16.

Depending on the desaturation pattern, two large classes of polyunsaturated fatty acids, the ω6 and the ω3 fatty acids, which differ with regard to their metabolism and their function, can be distinguished.

In the text which follows, polyunsaturated fatty acids are referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (poly unsaturated fatty acids, PUFA, long chain poly unsaturated fatty acids, LCPUFA).

The fatty acid linoleic acid ($18:2^{\Delta 9,12}$) acts as starting material for the ω6 metabolic pathway, while the ω3 pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed from linoleic acid by the activity of an ω3-desaturase (Tocher et al. (1998) Prog. Lipid Res. 37: 73-117; Domergue et al. (2002) Eur. J. Biochem. 269: 4105-4113).

Mammals, and thus also humans, have no corresponding desaturase activity (Δ12- and ω3-desaturase) for the formation of the starting materials and must therefore take up these fatty acids (essential fatty acids) via the food. Starting with these precursors, the physiologically important polyunsaturated fatty acids arachidonic acid (= ARA, $20:4^{\Delta 5,8,11,14}$), an ω6-fatty acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, $20:5^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA, $22:6^{\Delta 4,7,10,13,17,19}$) are synthesized via a sequence of desaturase and elongase reactions.

The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C_{20}- and C_{22}-PUFAs, respectively. This process proceeds via 4 steps. The first step is the condensation of malonyl-CoA onto the fatty acid acyl-CoA by ketoacyl-CoA synthase (KCS, hereinbelow referred to as elongase). This is followed by a reduction step (ketoacyl-CoA reductase, KCR), a dehydratation step (dehydratase) and a final reduction step (enoyl-CoA reductase). It has been postulated that the elongase activity affects the specificity and rate of the entire process (Millar and Kunst (1997) Plant Journal 12:121-131).

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and the pharmacological sector. Depending on whether they are free saturated or unsaturated fatty acids or else triacylglycerides with an elevated content of saturated or unsaturated fatty acids, they are suitable for very different applications. Thus, for example, lipids with unsaturated, specifically with polyunsaturated fatty acids, are preferred in human nutrition. The polyunsaturated ω3-fatty acids are supposed to have a positive effect on the cholesterol level in the blood and thus on the prevention of heart disease. The risk of heart disease, strokes or hypertension can be reduced markedly by adding these ω3-fatty acids to the food (Shimikawa (2001) World Rev. Nutr. Diet. 88: 100-108).

ω3-fatty acids also have a positive effect on inflammatory, specifically on chronically inflammatory, processes in association with immunological diseases such as rheumatoid arthritis (Calder (2002) Proc. Nutr. Soc. 61: 345-358; Cleland and James (2000) J. Rheumatol. 27: 2305-2307). They are therefore added to foodstuffs, specifically to dietetic foodstuffs, or are employed in medicaments. ω6-fatty acids such as arachidonic acid tend to have a negative effect in connection with these rheumatological diseases.

ω3- and ω6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomo-γ-linolenic acid, arachidonic acid and

eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG_2 series) which are formed from the ω6-fatty acids, generally promote inflammatory reactions, while eicosanoids (known as the PG_3 series) from ω3-fatty acids have little or no proinflammatory effect.

Polyunsaturated long-chain ω3-fatty acids such as eicosapentaenoic acid (= EPA, $C20:5^{\Delta5,8,11,14,17}$) or docosahexaenoic acid (= DHA, $C22:6^{\Delta4,7,10,13,16,19}$) are important components of human nutrition owing to their various roles in health aspects, including the development of the child brain, the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A (1995) Lipids 30:1-14; Horrocks, LA and Yeo YK (1999) Pharmacol Res 40:211-225).

Owing to the present-day composition of human food, an addition of polyunsaturated ω3-fatty acids, which are preferentially found in fish oils, to the food is particularly important. Thus, for example, polyunsaturated fatty acids such as docosahexaenoic acid (= DHA, $C22:6^{\Delta4,7,10,13,16,19}$) or eicosapentaenoic acid (= EPA, $C20:5^{\Delta5,8,11,14,17}$) are added to infant formula to improve the nutritional value. There is therefore a demand for the production of polyunsaturated long-chain fatty acids.

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella or Schizochytrium or from oil-producing plants such as soybeans, oilseed rape, and algae such as *Crypthecodinium* or *Phaeodactylum* and others, being obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, for example, fish. The free fatty acids are advantageously prepared by hydrolyzing the triacylglycerides. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (ARA, $C20:4^{\Delta5,8,11,14}$), dihomo-γ-linolenic acid (DHGL, $C20:3^{\Delta8,11,14}$) or docosapentaenoic acid (DPA, $C22:5^{\Delta7,10,13,16,19}$) are, however, not synthesized in oil crops such as oilseed rape, soybeans, sunflowers and safflower. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Owing to the positive characteristics of the polyunsaturated fatty acids, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of these fatty acids or triglycerides for the production of oils in various organisms with a modified content of

unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describe a Δ9-desaturase. WO 93/11245 claims a Δ15-desaturase and WO 94/11516 a Δ12-desaturase. Further desaturates are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al. (1990) J. Biol. Chem., 265: 20144-20149, Wada et al. (1990) Nature 347: 200-203 or Huang et al. (1999) Lipids 34: 649-659. However, the biochemical characterization of the various desaturases has been insufficient to date since the enzymes, being membrane-bound proteins, present great difficulty in their isolation and characterization (McKeon et al. (1981) Methods in Enzymol. 71: 12141-12147, Wang et al. (1988) Plant Physiol. Biochem., 26: 777-792).

As a rule, membrane-bound desaturases are characterized by being introduced into a suitable organism which is subsequently analyzed for enzyme activity by analyzing the starting materials and the products. Δ6-Desaturases are described in WO 93/06712, US 5,614,393, WO 96/21022, WO 00/21557 and WO 99/27111. The application of this enzyme for the production of fatty acids in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. The expression of various desaturases and the formation of polyunsaturated fatty acids is also described and claimed in WO 99/64616 or WO 98/46776. As regards the expression efficacy of desaturases and its effect on the formation of polyunsaturated fatty acids, it must be noted that the expression of a single desaturase as described to date has only resulted in low contents of unsaturated fatty acids/lipids such as, for example, γ-linolenic acid and stearidonic acid.

There have been a number of attempts in the past to obtain elongase genes. Millar and Kunst (1997) Plant Journal 12:121-131 and Millar et al. (1999) Plant Cell 11:825-838 describe the characterization of plant elongases for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the formation of waxes in plants (C_{28}-C_{32}). The synthesis of arachidonic acid and EPA is described, for example, in WO 01/59128, WO 00/12720, WO 02/077213 and WO 02/08401. The synthesis of polyunsaturated C24-fatty acids is described, for example, in Tvrdik et al. (2000) J. Cell Biol. 149:707-718 or in WO 02/44320.

Especially suitable microorganisms for the production of PUFAs are microalgae such as *Phaeodactylum tricornutum, Porphiridium species, Thraustochytrium species, Schizochytrium* species or *Crypthecodinium species*, ciliates such as *Stylonychia* or *Colpidium*, fungi such as

Mortierella, *Entomophthora* or *Mucor* and/or mosses such as *Physcomitrella*, *Ceratodon* and *Marchantia* (R. Vazhappilly & F. Chen (1998) Botanica Marina 41: 553-558; K. Totani & K. Oba (1987) Lipids 22: 1060-1062; M. Akimoto et al. (1998) Appl. Biochemistry and Biotechnology 73: 269-278). Strain selection has resulted in the development of a number of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFAs. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult process. Moreover, only limited amounts of the desired polyunsaturated fatty acids such as DPA, EPA or ARA can be produced with the aid of the abovementioned microorganisms; in addition, they are generally obtained as fatty acid mixtures. This is why recombinant methods are preferred whenever possible.

Higher plants comprise polyunsaturated fatty acids such as linoleic acid (C18:2) and linolenic acid (C18:3). ARA, EPA and DHA are found not at all in the seed oil of higher plants, or only in miniscule amounts (E. Ucciani: Nouveau Dictionnaire des Huiles Végétales [New Dictionary of the Vegetable Oils]. Technique & Documentation – Lavoisier, 1995. ISBN: 2-7430-0009-0). However, the production of LCPUFAs in higher plants, preferably in oil crops such as oilseed rape, linseed, sunflowers and soybeans, would be advantageous since large amounts of high-quality LCPUFAs for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically. To this end, it is advantageous to introduce, into oilseeds, genes which encode enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. These genes encode for example Δ6-desaturases, Δ6-elongases, Δ5-desaturases or Δ4-desaturases. These genes can advantageously be isolated from microorganisms and lower plants which produce LCPUFAs and incorporate them in the membranes or triacylglycerides. Thus, it has already been possible to isolate Δ6-desaturase genes from the moss *Physcomitrella patens* and Δ6-elongase genes from *P. patens* and from the nematode *C. elegans*.

Transgenic plants which comprise and express genes encoding LCPUFA biosynthesis enzymes and which, as a consequence, produce LCPUFAs have been described, for example, in DE-A-102 19 203 (process for the production of polyunsaturated fatty acids in plants). However, these plants produce LCPUFAs in amounts which require further optimization for processing the oils which are present in the plants. Thus, the ARA content in the plants described in DE-A-102 19

203 is only 0.4 to 2% and the EPA content only 0.5 to 1%, in each case based on the total lipid content of the plant.

To make possible the fortification of food and of feed with polyunsaturated, long-chain fatty acids, there is therefore a great need for a simple, inexpensive process for the production of polyunsaturated, long-chain fatty acids, specifically in plant systems.

One object of the invention is therefore to provide a process with which long-chain polyunsaturated fatty acids, especially eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid can be produced in large quantities and inexpensively in transgenic plants.

It has now surprisingly been found that the yield of long-chain polyunsaturated fatty acids, especially eicosapentaenoic, docosapentaenoic acid and/or docosahexaenoic acid, can be increased by expressing an optimized Δ5-elongase sequence in transgenic plants.

The PUFAs produced by the process of the invention comprise a group of molecules which higher animals are no longer able to synthesize and thus must consume, or which higher animals are no longer able to produce themselves in sufficient amounts and thus must consume additional amounts thereof, although they can easily be synthesized by other organisms such as bacteria.

Accordingly, the object of the invention is achieved by the process of the invention for producing eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in a transgenic plant, comprising the provision in the plant of at least one nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity,

where the nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified by comparison with the nucleic acid sequence in the organism from which the sequence is derived in that it is adapted to the codon usage in one or more plant species. To produce DHA it is additionally necessary to provide at least one nucleic acid sequence which codes for a polypeptide having a Δ4-desaturase activity in the plant.

The "provision in the plant" means in the context of the present invention that measures are taken so that the nucleic acid sequences coding for a polypeptide having a Δ6-desaturase activity,

a polypeptide having a Δ6-elongase activity, a polypeptide having a Δ5-desaturase activity and a polypeptide having a Δ5-elongase activity are present together in one plant. The "provision in the plant" thus comprises the introduction of the nucleic acid sequences into the plant both by transformation of a plant with one or more recombinant nucleic acid molecules which comprise said nucleic acid sequences, and by crossing suitable parent plants which comprise one or more of said nucleic acid sequences.

The nucleic acid sequence which codes for a polypeptide having a Δ5-elongase activity is modified according to the invention by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species. This means that the nucleic acid sequence has been specifically optimized for the purpose of the invention without the amino acid sequence encoded by the nucleic acid sequence having been altered thereby.

The genetic code is redundant because it uses 61 codons in order to specify 20 amino acids. Therefore, most of the 20 proteinogenic amino acids are therefore encoded by a plurality of triplets (codons). The synonymous codons which specify an individual amino acid are, however, not used with the same frequency in a particular organism; on the contrary there are preferred codons which are frequently used, and codons which are used more rarely. These differences in codon usage are attributed to selective evolutionary pressures and especially the efficiency of translation. One reason for the lower translation efficiency of rarely occurring codons might be that the corresponding aminoacyl-tRNA pools are exhausted and thus no longer available for protein synthesis.

In addition, different organisms prefer different codons. For this reason, for example, the expression of a recombinant DNA derived from a mammalian cell frequently proceeds only suboptimally in *Escherichia coli* (*E. coli*) cells. It is therefore possible in some cases to increase expression by replacing rarely used codons with frequently used codons. Without wishing to be bound to one theory, it is assumed that the codon-optimized DNA sequences make more efficient translation possible, and the mRNAs formed therefrom possibly have a greater half-life in the cell and therefore are available more frequently for translation. From what has been said above, it follows that codon optimization is necessary only if the organism in which the nucleic acid

sequence is to be expressed differs from the organism from which the nucleic acid sequence is originally derived.

For many organisms of which the DNA sequence of a relatively large number of genes is known there are tables from which the frequency of use of particular codons in the respective organism can be taken. It is possible with the aid of these tables to translate protein sequences with relatively high accuracy back into a DNA sequence which comprises the codons preferred in the respective organism for the various amino acids of the protein. Tables on codon usage can be found inter alia at the following Internet address: kazusa.or.ip/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelechon (Software Leto) or Geneart (Software GeneOptimizer).

Adaptation of the sequences to the codon usage in a particular organism can take place with the aid of various criteria. On the one hand, it is possible to use for a particular amino acid always the codon which occurs most frequently in the selected organism but, on the other hand, the natural frequency of the various codons can also be taken into account, so that all the codons for a particular amino acid are incorporated into the optimized sequence according to their natural frequency. Selection of the position at which a particular base triplet is used can take place at random in this case. The DNA sequence was adapted according to the invention taking account of the natural frequency of individual codons, it also being suitable to use the codons occurring most frequently in the selected organism.

It is particularly preferred for a nucleic acid sequence from *Ostreococcus tauri* which codes for a polypeptide having a Δ5-elongase activity, such as, for example, the polypeptide depicted in SEQ ID NO: 110, to be adapted at least to the codon usage in oilseed rape, soybean and/or flax. The nucleic acid sequence originally derived from *Ostreococcus tauri* is preferably the sequence depicted in SEQ ID NO: 109. The DNA sequence coding for the Δ5-elongase is adapted in at least 20% of the positions, preferably in at least 30% of the positions, particularly preferably in at least 40% of the positions and most preferably in at least 50% of the positions to the codon usage in oilseed rape, soybean and/or flax.

The nucleic acid sequence used is most preferably the sequence indicated in SEQ ID NO: 64.

It will be appreciated that the invention also encompasses those codon-optimized DNA sequences which code for a polypeptide having the activity of a Δ5-elongase and whose amino

acid sequence is modified in one or more positions by comparison with the wild-type sequence but which still has substantially the same activity as the wild-type protein.

The nucleic acid sequence which codes for a polypeptide having a Δ6-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41, preferably having the sequence depicted in SEQ ID NO: 1,b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 or 42, preferably in SEQ ID SEQ ID NO: 2,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated a) or b) above, in particular of the nucleic acid sequence indicated in SEQ ID NO: 1, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the sequence indicated in SEQ ID NO: 1, and

e) nucleic acid sequences which code for an amino acid sequence and which have at least one, for example 2, 3, 4, 5, 6, 7 or 8, preferably all of the amino acid pattern indicated in SEQ ID NO: 43, 44, 45, 46, 47, 48, 49 or 50.

Amino acid pattern means short amino acid sequences which preferably comprise less than 50, particularly preferably less than 40 and especially from 10 to 40 and even more preferably from 10 to 30 amino acids.

For the present invention, the identity is ascertained preferably over the full length of the nucleotide or amino acid sequences of the invention, for example for the nucleic acid sequence indicated in SEQ ID NO: 64 over the full length of 903 nucleotides.

The nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 171, 173, 175, 177, 179, 181 or 183, especially having the sequence depicted in SEQ ID NO: 171,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 172, 174, 176, 178, 180, 182 or 184, especially for the amino acid sequence indicated in SEQ ID NO: 172,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 1, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the sequence indicated in SEQ ID NO: 171, and

e) nucleic acid sequences which code for an amino acid sequence and which have at least one, for example 2, 3, 4, 5, 6, 7 or 8, preferably all of the amino acid pattern indicated in SEQ ID NO: 185, 186, 187, 188, 189, 190, 191 or 192.

The nucleic acid sequence which codes for a polypeptide having a Δ6-elongase activity is in particular likewise a codon-optimized sequence according to the present invention, preferably the nucleic acid sequence depicted in SEQ ID NO: 122.

The nucleic acid sequence which codes for a polypeptide having a Δ5-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 51, 53 or 55, preferably having the sequence depicted in SEQ ID NO: 51,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 52, 54 or 56, preferably for the amino acid sequence indicated in SEQ ID NO: 52,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated in a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 51, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the nucleic acid sequences indicated in a) or b) above, especially to the nucleic acid indicated under SEQ ID NO: 51, and

e) nucleic acid sequences which code for an amino acid sequence which have at least one, for example 2, 3, 4, 5, 6 or 7, preferably all of the amino acid pattern indicated in SEQ ID NO: 57, 58, 59, 60, 61, 62 or 63.

Further suitable nucleic acid sequences can be found by the skilled worker from the literature or the well-known gene libraries such as, for example, ncbi.nlm.nih.gov.

In a further preferred embodiment of the process, additionally one or more nucleic acid sequences which code for a polypeptide having the activity of an ω-3-desaturase and/or of a Δ4-desaturase are introduced into the plant.

The nucleic acid sequence which codes for a polypeptide having an ω-3-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 193 or 195, preferably the sequence depicted in SEQ ID NO: 193,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 194,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequence indicated in SEQ ID NO: 193 or 195 under stringent conditions, and

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95%, and especially at least 96%, 97%, 98% or 99%, identical to the sequence indicated in SEQ ID NO: 193 or 195.

The ω-3-desaturase advantageously used in the process of the invention makes it possible to shift from the ω-6 biosynthetic pathway to the ω-3 biosynthetic pathway, leading to a shift from $C_{18:2}$ to $C_{18:3}$ fatty acids. It is further advantageous for the ω-3-desaturase to convert a wide range of phospholipids such as phosphatidylcholine (= PC), phosphatidylinositol (= PIS) or

phosphatidylethanolamine (= PE). Finally, desaturation products can also be found in the neutral lipids (= NL), that is to say in the triglycerides.

The nucleic acid sequence which codes for a polypeptide having a Δ4-desaturase activity is preferably selected from the group consisting of:

a) nucleic acid sequences having the sequence depicted in SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, preferably having the sequence depicted in SEQ ID NO: 77,

b) nucleic acid sequences which code for the amino acid sequence indicated in SEQ ID NO: 78, 80, 82, 84, 86, 88, 90, 92 or 94, preferably for the amino acid sequence indicated in SEQ ID NO: 78,

c) nucleic acid sequences which hybridize with the complementary strand of the nucleic acid sequences indicated in a) or b) above, especially of the nucleic acid sequence indicated in SEQ ID NO: 77, under stringent conditions,

d) nucleic acid sequences which are at least 60%, 65%, 70%, 75% or 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%, particularly preferably at least 91%, 92%, 93%, 94% or 95% and especially at least 96%, 97%, 98% or 99%, identical to the sequence indicated in SEQ ID NO: 77, and

e) nucleic acid sequences which code for an amino acid sequence which have at least one, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14, preferably all of the amino acid pattern indicated in SEQ ID NO: 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

The Δ4-desaturase which is advantageously used in the process of the invention catalyzes the introduction of a double bond into the fatty acid docosapentaenoic acid, leading to formation of docosahexaenoic acid.

It is advantageous for the described process of the invention additionally to introduce further nucleic acids which code for enzymes of fatty acid or lipid metabolism into the plants in addition to the nucleic acid sequences which code for polypeptides having a Δ6-desaturase activity, a Δ6-elongase activity, a Δ5-desaturase activity and a Δ5-elongase activity, and to the nucleic acid sequences which are introduced if appropriate and which code for a polypeptide having an ω-3-desaturase activity and/or a Δ4-desaturase activity.

It is possible in principle to use all genes of fatty acid or lipid metabolism in combination with the nucleic acid sequences used in the process of the invention; genes of fatty acid or lipid metabolism selected from the group of acyl-CoA dehydrogenase(s), acyl-ACP (acyl carrier protein) desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s),acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) are preferably used in combination with the Δ6-elongase, Δ6-desaturase, Δ5-desaturase and the Δ5-elongase, and if appropriate the ω3-desaturase and/or the Δ4-desaturase, it being possible to use individual genes or a plurality of genes in combination.

The nucleic acids used in the process of the invention are advantageously expressed in vegetative tissues (somatic tissue). Vegetative tissue means in the context of this invention a tissue which is propagated through mitotic divisions. Tissue of this type also arises through asexual reproduction (apomixis) and propagation. Propagation is the term used when the number of individuals increases in consecutive generations. These individuals arising through asexual propagation are very substantially identical to their parents. Examples of such tissues are leaf, flower, root, stalk, runners above or below ground (side shoots, stolons), rhizomes, buds, tubers such as root tubers or stem tubers, bulb, brood bodies, brood buds, bulbuls or turion. Such tissues may also arise through pseudo vivipary, true vivipary or vivipary caused by humans. However, seeds arising through agamospermy, as are typical of Asteraceae, Poaceae or Rosaceae, are also included among the vegetative tissues in which expression advantageously takes place. The nucleic acids used in the process of the invention are expressed to a small extent or not at all in generative tissue (germ line tissue). Examples of such tissues are tissues arising through sexual reproduction, i.e. meiotic cell divisions, such as, for example, seeds arising through sexual processes.

A small extent means that, compared with vegetative tissue, the expression measured at the RNA and/or protein level is less than 5%, advantageously less than 3%, particularly advantageously less than 2%, most preferably less than 1; 0.5; 0.25 or 0.125%.

The nucleic acid sequences are particularly preferably expressed in the leaves of the transgenic plants. This has the advantage that the LCPUFAs produced according to the invention can be

taken in by animals and humans directly by consuming the leaves, and no previous processing of the plant material is necessary.

Expression of the nucleic acid sequences of the invention in the leaf can be achieved by using constitutive or leaf-specific promoters.

"Constitutive promoters" are promoters which make expression possible in a large number of, preferably in all, tissues over a substantial period during plant development, preferably throughout plant development. A promoter from a plant or from a plant virus is preferably used. The promoter of the CaMV (cauliflower mosaic virus) 35S transcript (Franck et al. (1980) Cell 21: 285-294), the 19S CaMV promoter (US 5,352,605), the actin promoter from rice (McElroy et al. (1990) Plant Cell 2: 163-171), the legumin B promoter (GenBank Acc. No. X03677), the agrobacterium nopaline synthase promoter, the TR dual promoter, the agrobacterium octopine synthase promoter, the ubiquitin promoter (Holtorf et al. (1995) Plant Mol. Biol. 29: 637-649), the Smas promoter, the cinnamoyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits, the pEMU promoter (Last et al. (1991) Theor. Appl. Genet. 81: 581-588), the MAS promoter (Velten et al. (1984) EMBO J. 3(12): 2723-2730), the histone H3 promoter from corn (Lepetit et al. (1992) Mol. Gen. Genet. 231: 276-285), the promoter of the nitrilase 1 gene from arabidopsis (GenBank Acc. No. U38846, nucleotides 3862-5325) and the promoter of a proline-rich protein from wheat (WO 91/13991) and further promoters which mediate constitutive gene expression. The promoter of the CaMV 35S transcript is particularly preferred.

It is in principle possible to use all naturally occurring constitutive promoters with their regulatory sequences like those mentioned above for the novel process. However, it is likewise possible to use synthetic promoters in addition or alone.

"Leaf-specific promoters" are promoters which show a high activity in the leaf and no or only low activity in other tissues. "Low activity" means in the context of the invention that the activity in other tissues is less than 20%, preferably less than 10%, particularly preferably less than 5% and most preferably less than 3, 2 or 1% of the activity in the leaf. Examples of suitable leaf-specific promoters are the promoters of the small subunit of rubisco (Timko et al. (1985) Nature 318: 579-582) and of the chlorophyll a/b-binding protein (Simpson et al. (1985) EMBO J. 4: 2723-2729).

The skilled worker is aware of further leaf-specific promoters, or he can isolate further suitable promoters with known methods. Thus, the skilled worker is able to identify leaf-specific regulatory nucleic acid elements with the aid of conventional methods of molecular biology, e.g. hybridization experiments or DNA-protein binding studies. This entails for example in a first step isolating the total poly(A)$^+$ RNA from leaf tissue of the desired organism from which the regulatory sequences are to be isolated, and setting up a cDNA library. In a second step, cDNA clones which are based on poly(A)$^+$ RNA molecules from a non-leaf tissue are used to identify, by means of hybridization, those clones from the first library whose corresponding poly(A)$^+$ RNA molecules accumulate only in leaf tissue. Subsequently, these cDNAs identified in this way are used to isolate promoters which have leaf-specific regulatory elements. Further PCR-based methods for isolating suitable leaf-specific promoters are additionally available to the skilled worker.

It is, of course, also possible for the nucleic acid sequences of the present invention to be expressed in the seeds of the transgenic plants by using seed-specific promoters which are active in the embryo and/or in the endosperm. Seed-specific promoters can in principle be isolated both from dicotyledonous and from monocotyledonous plants. Preferred promoters are listed hereinafter: USP (unknown seed protein) and vicilin (*Vicia faba*) (Bäumlein et al. (1991) Mol. Gen Genet. 225(3): 459-467), napin (oilseed rape) (US 5,608,152), conlinin (flax) (WO 02/102970), acyl-carrier protein (oilseed rape) (US 5,315,001 and WO 92/18634), oleosin (*Arabidopsis thaliana*) (WO 98/45461 and WO 93/20216), phaseolin (*Phaseolus vulgaris*) (US 5,504,200), Bce4 (WO 91/13980), legume B4 (LegB4 promoter) (Bäumlein et al. (1992) Plant J. 2(2): 233-239), Lpt2 and lpt1 (barley) (WO 95/15389 and WO 95/23230), seed-specific promoters from rice, corn and wheat (WO 99/16890), Amy32b, Amy 6-6 and aleurain (US 5,677,474), Bce4 (oilseed rape) (US 5,530,149), glycinin (soybean) (EP 571 741), phosphoenolpyruvate carboxylase (soybean) (JP 06/62870), ADR 12-2 (soybean) (WO 98/08962), isocitrate lyase (oilseed rape) (US 5,689,040) or α-amylase (barley) (EP 781 849).

In a particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid

sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of produced LCPUFAs, especially of the C22 fatty acids, in the seed oil is at least 5% by weight, advantageously at least 6, 7, 8, 9 or 10% by weight, preferably at least 11, 12, 13, 14 or 15% by weight, particularly preferably at least 16, 17, 18, 19 or 20% by weight, very particularly preferably at least 25, 30, 35 or 40% by weight, of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of C22 fatty acids in the seed oil is at least 8% by weight of the seed oil content.

In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of docosahexaenoic acid in the seed oil is at least 1% by weight, preferably at least 1.1, 1.2, 1.3, 1.4 or 1.5% by weight, particularly preferably at least 1.6, 1.7, 1.8 or 1.9% by weight, especially at least 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8 or 2.9% by weight, further preferably at least 3, 3.5 or 4% by weight of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of docosahexaenoic acid in the seed oil is at least 1.9% by weight of the seed oil content. It is known to the skilled worker in this connection that to produce docosahexaenoic acid additionally one or more nucleic acid sequences which codes for a polypeptide having the activity of a Δ4-desaturase activity are required. A nucleic acid sequence which codes for a polypeptide having the activity of a Δ4-desaturase activity is advantageously selected from the group consisting of nucleic acid sequences having the sequence depicted in SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, preferably having the sequence depicted in SEQ ID NO: 77.

In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a Δ5-elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates by being adapted to the codon usage in one or more plant species, preferably the nucleic acid sequence described in SEQ ID NO: 64, are expressed in generative tissue, especially in the seed. Specific expression in the seed advantageously takes place by using one of the abovementioned seed-specific promoters, especially using the napin promoter. In this particularly preferred embodiment, the content of docosahexaenoic acid in the seed oil is at least 1% by weight, preferably at least 1.1, 1.2, 1.3, 1.4 or 1.5% by weight, particularly preferably at least 1.6, 1.7, 1.8 or 1.9% by weight, especially at least 2, 2.1, 2.2, 2.5, 2.6, 2.7, 2.8 or 2.9% by weight, further preferably at least 3, 3.5 or 4% by weight of the seed oil content. In this case, the content of the produced LCPUFAs, especially of the C22 fatty acids, in the seed oil is at least 5% by weight, advantageously at least 6, 7, 8, 9 or 10% by weight, preferably at least 11, 12, 13, 14 or 15% by weight, particularly preferably at least 16, 17, 18, 19 or 20% by weight, very particularly preferably at least 25, 30, 35 or 40% by weight of the seed oil content. In a further particularly preferred embodiment with the nucleic acid sequence described in SEQ ID NO: 63, the content of docosahexaenoic acid in the seed oil is at least 1.9% by weight of the seed oil content, with the content of C22 fatty acids in the seed oil being at least 8% by weight of the seed oil content.

Plant gene expression can also be achieved via a chemically inducible promoter (see a review in Gatz (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that the gene expression takes place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

Promoters which respond to biotic or abiotic stress conditions are also suitable, for example the pathogen-induced PRP1 gene promoter (Ward et al., Plant. Mol. Biol. 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the chill-inducible potato alpha-amylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Other promoters which are also particularly suitable are those which bring about the plastid-specific expression, since plastids constitute the compartment in which precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters, such as the viral RNA polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the Arabidopsis clpP promoter, described in WO 99/46394.

It will be appreciated that the polyunsaturated fatty acids produced according to the invention can be produced not only in intact transgenic plants but also in plant cell cultures or in callous cultures.

The polyunsaturated fatty acids produced in the process are advantageously bound in phospholipids and/or triacylglycerides, but may also occur as free fatty acids or else bound in the form of other fatty acid esters in the organisms. They may in this connection be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different phospholipids such as phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and/or phosphatidylserine and/or triacylglycerides, monoacyl-glycerides and/or diacylglycerides. The LCPUFAs EPA, DPA and DHA produced in the process are advantageously present in phosphatidylcholine and/or phosphatidylethanolamine and/or in the triacylglycerides. The triacylglycerides may additionally also comprise further fatty acids such as short-chain fatty acids having 4 to 6 C atoms, medium-chain fatty acids having 8 to 12 C atoms or long-chain fatty acids having 14 to 24 C atoms. They preferably comprise long-chain fatty acids, particularly preferably C_{20} or C_{22} fatty acids.

The term "glyceride" is understood as meaning glycerol esterified with one, two or three carboxyl radicals (mono-, di- or triglyceride). "Glyceride" is also understood as meaning a mixture of various glycerides. The glyceride is preferably a triglyceride. The glyceride or glyceride mixture can comprise further additions, for example free fatty acids, antioxidants, proteins, carbohydrates, vitamins and/or other substances.

A"glyceride" for the purposes of the process according to the invention is furthermore understood as meaning derivatives which are derived from glycerol. In addition to the above-described fatty acid glycerides, these also include glycerophospholipids and glyceroglycolipids. Preferred examples which may be mentioned here are the glycerophospholipids such as lecithin

(phosphatidylcholine), cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylacylglycerophospholipids.

Phospholipids are to be understood as meaning, for the purposes of the invention, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and/or phosphatidylinositol.

The fatty acid esters with polyunsaturated C_{18}-, C_{20}- and/or C_{22}-fatty acid molecules can be isolated in the form of an oil or lipid, for example in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three or four, preferably four, five or six double bonds, from the useful plants which have been used for the preparation of the fatty acid esters; advantageously, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of the phosphatidyl ester, especially preferably in the form of the triacylglycerides, phosphatidylcholine and/or phosphatidylethanolamine. In addition to these esters, the polyunsaturated fatty acids are also present in the plants as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

The LCPUFAs produced in the process of the invention are produced with a content of at least 4% by weight, advantageously of at least 5, 6, 7, 8, 9 or 10% by weight, preferably of at least 11, 12, 13, 14 or 15% by weight, particularly preferably of at least 16, 17, 18, 19, or 20% by weight, very particularly preferably of at least 25, 30, 35 or 40% by weight based on the total fatty acids in the transgenic plant. The fatty acids EPA, DPA and/or DHA produced in the process of the invention are moreover present with a content of in each case at least 5% by weight, preferably of in each case at least 6, 7, 8 or 9% by weight, particularly preferably of in each case at least 10,

11 or 12% by weight, most preferably of in each case at least 13, 14, 15, 16, 17, 18, 19 or 20% by weight based on the total fatty acids in the transgenic plant.

The fatty acids are advantageously produced in bound form. It is possible with the aid of the nucleic acids used in the process of the invention for these unsaturated fatty acids to be put on the sn1, sn2 and/or sn3 position of the advantageously produced triacylglycerides. Advantageously, at least 11% of the triacylglycerides are doubly substituted (meaning on the sn1 and sn2 or sn2 and sn3 positions). Triply substituted triacylglycerides are also detectable. Since a plurality of reaction steps take place from the starting compounds linoleic acid (C18:2) and linolenic acid (C18:3), the final products of the process, such as, for example, arachidonic acid (ARA) or eicosapentaenoic acid (EPA), do not result as absolute pure products; traces or larger amounts of the precursors are always also present in the final product. If, for example, both linoleic acid and linolenic acid are present in the initial plant, the final products such as ARA or EPA and/or DPA and/or DHA are also present as mixtures. The precursors should advantageously amount to not more than 20% by weight, preferably not more than 15% by weight, particularly preferably not as 10% by weight, very particularly preferably not more than 5% by weight based on the amount of the respective final product. Advantageously, only ARA or EPA and/or DPA and/or DHA are produced in the process of the invention, bound or as free acids, as final products in a transgenic plant.

Fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise 6 to 15% palmitic acid, 1 to 6% stearic acid; 7-85% oleic acid; 0.5 to 8% vaccenic acid, 0.1 to 1% arachic acid, 7 to 25% saturated fatty acids, 8 to 85% monounsaturated fatty acids and 60 to 85% polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Preferably at least 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 or 1% arachidonic acid in the total fatty acid content, are present as advantageous polyunsaturated fatty acid in the fatty acid ester or fatty acid mixtures. The fatty acid esters or fatty acid mixtures produced by the process of the invention further advantageously comprise fatty acids selected from the group of fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enonic acid), malvalic acid (8,9-methyleneheptadec-8-enonic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienonic acid), vernonic acid (9,10-epoxyoctadec-12-enonic acid), taric acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynonic

acid, pyrulic acid (t10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynonic acid, petroselenic acid (cis-6-octadecenonic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid, catalpic acid (9t11t13c-octadecatrienoic acid), eleosteric acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienonic acid). In general, the aforementioned fatty acids are advantageously present only in traces in the fatty acid esters or fatty acid mixtures produced by the process of the invention, meaning that their occurrence, based on the total fatty acid content, is less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, particularly preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very particularly preferably less than 4%, 3%, 2% or 1%. In a further preferred form of the invention the occurrence of these aforementioned fatty acids, based on the total fatty acids, is less than 0.9%; 0.8%; 0.7%; 0.6% or 0.5%, particularly preferably less than 0.4%; 0.3%; 0.2%; 0.1%. The fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise less than 0.1% based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, C23:6$^{\Delta 3,8,12,15,18,21}$).

It is possible through the nucleic acid sequences used in the process of the invention to achieve an increase in the yield of LCPUFAs in the transgenic plants of at least 50%, advantageously of at least 80%, particularly advantageously of at least 100%, very particularly advantageously of at least 150%, compared with the non-transgenic plants.

Chemically pure polyunsaturated fatty acids or fatty acid compositions can also be synthesized by the processes described above. To this end, the fatty acids or the fatty acid compositions are isolated from the plants in the known manner, for example via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

In principle, all dicotyledonous or monocotyledonous useful plants are suitable for the process of the invention. Useful plants mean plants which serve to produce foods for humans and animals,

to produce other consumables, fibers and pharmaceuticals, such as cereals, e.g. corn, rice, wheat, barley, millet, oats, rye, buckwheat; such as tubers, e.g. potato, cassava, sweet potato, yams etc.; such as sugar plants e.g. sugarcane or sugarbeet; such as legumes, e.g. beans, peas, broad bean etc.: such as oil and fat crops, e.g. soybean, oilseed rape, sunflower, safflower, flax, camolina etc., to mention only a few. Advantageous plants are selected from the group of plant families consisting of the families of *Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreacea, Elaeagnaceae, Ericceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae* and *Valerianaceae*.

Examples which may be mentioned are the following plants: *Anacardiaceae* such as the genera *Pistacia, Mangifera, Anacardium*, for example the genus and species *Pistacia vera* [pistachio], *Mangifer indica* (mango) or *Anacardium occidentale* (cashew), Asteraceae such as the genera *Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana*, e.g. the genus and species Calendula officinalis (common marigold), *Carthamus tinctorius* (safflower), *Centaurea cyanus* (cornflower), *Cichorium intybus* (chicory), *Cynara scolymus* (artichoke), *Helianthus annus* (sunflower), *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola* L. ssp. sativa, *Lactus scariola* L. var. integrata, *Lactuca scariola* L. var. integrifolia, *Lactuca sativa* subsp. romana, *Locusta communis, Valeriana locusta* (lettuce), *Tagetes lucida, Tagetes erecta* or *Tagetes tenuifolia* (French marigold), *Apiaceae* such as the genus *Daucus*, e.g. the genus and species *Daucus carota* (carrot), *Betulaceae* such as the genus *Corylus*, e.g. the genera and species *Corylus avellana* or *Corylus colurna* (hazelnut), *Boraginaceae* such as the genus Borago, e.g. the genus and species Borago officinalis (borage), *Brassicaceae* such as the genera *Brassica, Camelina, Melanosinapis, Sinapis, Arabadopsis*, e.g. the genera and species *Brassica napus, Brassica rapa* ssp. (oilseed rape), *Sinapis arvensis Brassica juncea, Brassica juncea* var. juncea, *Brassica juncea* var. crispifolia, *Brassica juncea* var. foliosa, *Brassica nigra, Brassica sinapioides, Camelina sativa, Melanosinapis communis* (mustard), *Brassica oleracea* (feed beet) or *Arabidopsis thaliana*, *Bromeliaceae* such as the genera *Anana, Bromelia* (pineapple), e.g. the genera and species *Anana comosus, Ananas ananas*

or *Bromelia comosa* (pineapple), *Caricacea*e such as the genus *Carica* such as the genus and species *Carica papaya* (papaya), Cannabaceae such as the genus Cannabis such as the genus and species *Cannabis sative* (hemp), *Convolvulaceae* such as the genera *Ipomoea, Convolvulus*, e.g. the genera and species *Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba* or Convolvulus panduratus (sweet potato, batate), *Chenopodiaceae* such as the genus *Beta* such as the genera and species *Beta vulgaris, Beta vulgaris* var. altissima, *Beta vulgaris* var. vulgaris, *Beta maritima, Beta vulgaris* var. perennis, *Beta vulgaris* var. conditiva or *Beta vulgaris* var. esculenta (sugarbeet), *Cucurbitaceae* such as the genus *Cucubita*, e.g. the genera and species Cucurbita maxima, *Cucurbita mixta, Cucurbita pepo* or *Cucurbita moschata* (pumpkin), *Elaeagnacea*e such as the genus *Elaeagnus*, e.g. the genus and species *Olea europaea* (olive), *Ericaceae* such as the genus *Kalmia*, e.g. the genera and species *Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros* or Kalmia lucida (mountain laurel), Euphorbiaceae such as the genera *Manihot, Janipha, Jatropha, Ricinus*, e.g. the genera and species *Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta* (cassava) or *Ricinis communis* (castor oil plant), *Fabaceae* such as the genera *Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja*, e.g. the genera and species *Pisum sativum, Pisum arvense, Pisum humile* (pea), *Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa* (acacia), *Medicago sativa, Medicago falcata, Medicago varia* (alfalfa), *Glycine max, Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida* or Soja max (soybean), Geraniaceae such as the genera *Pelargonium, Cocos, Oleum*, e.g. the genera and species *Cocos nucifera, Pelargonium grossularioides or Oleum cocois* (coconut), Gramineae such as the genus Saccharum, e.g. the genus and species *Saccharum officinarum, Juglandaceae* such as the genera *Juglans, Wallia*, e.g. the genera and species *Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans*

cinerea, *Wallia cinerea*, *Juglans bixbyi*, *Juglans californica*, *Juglans hindsii*, *Juglans intermedia*, *Juglans jamaicensis*, *Juglans major*, *Juglans microcarpa*, *Juglans nigra* or *Wallia nigra* (walnut), *Lauraceae* such as the genera *Persea*, *Laurus*, e.g. the genera and species *Laurus nobilis* (bay), *Persea americana*, *Persea gratissima* or *Persea persea* (avocado), *Leguminosae* such as the genus *Arachis*. e.g. the genus and species Arachis hypogaea (peanut), Linaceae such as the genera *Linum*, *Adenolinum*, e.g. the genera and species *Linum usitatissimum*, *Linum humile*, *Linum austriacum*, *Linum bienne*, *Linum angustifolium*, *Linum catharticum*, *Linum flavum*, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum lewisii*, *Linum narbonense*, *Linum perenne*, *Linum perenne* var. lewisii, *Linum pratense* or *Linum trigynum* (flax), *Lythrarieae* such as the genus *Punica*, e.g. the genus and species *Punica granatum* (pomegranate), *Malvaceae* such as the genus *Gossypium*, e.g. the genera and species *Gossypium hirsutum*, *Gossypium arboreum*, *Gossypium barbadense*, *Gossypium herbaceum* or *Gossypium thurberi* (cotton), *Musaceae* such as the genus *Musa*, e.g. the genera and species *Musa nana*, *Musa acuminata*, *Musa paradisiaca*, *Musa* spp. (banana), *Onagraceae* such as the genera *Camissonia*, *Oenothera*, e.g. the genera and species *Oenothera biennis* or *Camissonia brev*ipes (evening primrose), *Palmae* such as the genus *Elaeis*, e.g. the genus and species *Elaeis guineensis* (oil palm), *Papaveraceae* such as the genus *Papaver*, e.g. the genera and species *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium* (poppy), *Pedaliaceae* such as the genus *Sesamum* e.g the genus and species *Sesamum indicum* (sesame), *Piperaceae* such as the genera *Piper*, *Artanthe*, *Peperomia*, *Steffensia*, e.g. the genera and species *Piper aduncum*, *Piper amalago*, *Piper angustifolium*, *Piper auritum*, *Piper betel*, *Piper cubeba*, *Piper longum*, *Piper nigrum*, *Piper retrofractum*, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata* (cayenne pepper), *Poaceae* such as the genera *Hordeum*, *Secale*, *Avena*, *Sorghum*, *Andropogon*, *Holcus*, *Panicum*, *Oryza*, *Zea* (corn), *Triticum*, e.g. the genera and species *Hordeum vulgare*, *Hordeum jubatum*, *Hordeum murinum*, *Hordeum secalinum*, *Hordeum distichon*, *Hordeum aegiceras*, *Hordeum hexastichon*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, *Hordeum secalinum* (barley), Secale cereale (rye), *Avena sativa*, *Avena fatua*, *Avena byzantina*, *Avena fatua* var. sativa, *Avena hybrida* (oats), *Sorghum bicolor*, *Sorghum halepense*, *Sorghum saccharatum*, *Sorghum vulgare*, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum*

durra, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*, *Holcus halepensis*, *Sorghum miliaceum*, *Panicum militaceum* (millet), *Oryza sativa*, *Oryza latifolia* (rice), *Zea mays* (corn), *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare* (wheat), *Porphyridiaceae* such as the genera *Chroothece*, *Flintiella*, *Petrovanella*, *Porphyridium*, *Rhodella*, *Rhodosorus*, *Vanhoeffenia*, e.g. the genus and species *Porphyridium cruentum*, *Proteaceae* such as the genus *Macadamia*, e.g. the genus and species *Macadamia intergrifolia* (macadamia), *Rubiaceae* such as the genus *Coffea*, e.g. the genera and species *Coffea* spp., *Coffea arabica*, *Coffea canephora* or *Coffea liberica* (coffee), *Scrophulariaceae* such as the genus *Verbascum*, e.g. the genera and species *Verbascum blattaria*, *Verbascum chaixii*, *Verbascum densiflorum*, *Verbascum lagurus*, *Verbascum longifolium*, *Verbascum lychnitis*, *Verbascum nigrum*, *Verbascum olympicum*, *Verbascum phlomoides*, *Verbascum phoenicum*, *Verbascum pulverulentum* or *Verbascum thapsus* (mullein), *Solanaceae* such as the genera *Capsicum*, *Nicotiana*, *Solanum*, *Lycopersicon*, e.g. the genera and species *Capsicum annuum*, *Capsicum annuum* var. glabriusculum, *Capsicum frutescens* (pepper), Capsicum annuum (paprika), *Nicotiana tabacum*, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana glauca*, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*, *Nicotiana quadrivalvis*, *Nicotiana repanda*, *Nicotiana rustica*, *Nicotiana sylvestris* (tobacco), *Solanum tuberosum* (potato), *Solanum melongena* (aubergine), *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*, *Solanum integrifolium* or *Solanum lycopersicum* (tomato), *Sterculiaceae* such as the genus *Theobroma*, e.g. the genus and species *Theobroma cacao* (cocoa), or *Theaceae* such as the genus *Camellia*, e.g. the genus and species *Camellia sinensis* (tea).

In an advantageous embodiment of the process, the useful plants used are oil fruit plants which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower (*Carthamus tinctoria*), poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, verbascum, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, flax, soybean, pistachios, borage, trees (oil palm, coconut or walnut) or arable crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, *Tagetes*, *Solanaceae* plants such as potato, tobacco, egg plant and tomato, *Vicia* species, pea, alfalfa or bushy plants (coffee, cacao, tea), *Salix* species, and perennial grasses and

fodder crops. Advantageous plants according to the invention are oil fruit plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, *Calendula, Punica*, evening primrose, pumpkin/squash, flax, soybean, borage, trees (oilpalm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as sunflower, safflower, tobacco, verbascum, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, flax, hemp or thistle. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, flax, or hemp.

It is also advantageous to express the nucleic acid sequences of the invention in the leaves of feed or food plants and thus to increase the content of eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid in the leaves. Preferred feed plants are, for example, trefoil species such as red clover (*Trifolium pratense*), white clover (*Trifolium repens*), alsike clover (*Trifolium hybridum*), sainfoin (*Onobrychis viccifolia*), Egyptian clover (*Trifolium alexandrinium*) and Persian clover (*Trifolium resupinatum*). Preferred food plants are for instance lettuce species such as *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola* L. ssp. sativa, *Lactuca scariola* L. var. integrata, *Lactuca scariola* L. var. integrifolia, *Lactuca sativa* subsp. romana, *Locusta communis* and *Valeriana locusta*.

It is possible through the enzymatic activity of the nucleic acid sequences which are used in the process of the invention and which code for polypeptides having Δ6-elongase, Δ6-desaturase, Δ5-desaturase and/or Δ5-elongase activity, advantageously in combination with nucleic acid sequences which code for polypeptides having ω3-desaturase and/or Δ4-desaturase activity, and further nucleic acid sequences which code for polypeptides of fatty acid or lipid metabolism, such as further polypeptides having Δ5-, Δ6-, Δ8-, Δ12-desaturase or Δ5-, Δ6- or Δ9-elongase activity, to produce a wide variety of polyunsaturated fatty acids in the process of the invention. Depending on the useful plants chosen for use in the process of the invention, mixtures of the various polyunsaturated fatty acids or individual polyunsaturated fatty acids such as EPA, DPA or DHA can be produced in free or bound form. Depending on the prevailing fatty acid composition in the starting plant (C18:2 or C18:3 fatty acids), the resulting fatty acids are derived from C18:2 fatty acids, such as GLA, DGLA or ARA or are derived from C18:3 fatty acids, such as EPA, DPA or DHA. If the only unsaturated fatty acid present in the plant used for the process is linoleic acid (LA, C18:$2^{\Delta 9,12}$), the only possible products of the process are GLA,

DGLA and ARA, which may be present as free fatty acids or bound. If the only unsaturated fatty acid present in the plant used in the process is α-linolenic acid (ALA, C18:3$^{\Delta 9,12,15}$), for example as in flax, the only possible products of the process are SDA, ETA, EPA, DPA and/or DHA, which may be present as described above as free fatty acids or bound. It is possible to produce in a targeted manner only individual products in the plant by modifying the activity of the enzymes used in the process and involved in the synthesis Δ6-elongase, Δ6-desaturase, Δ5-desaturase and/or Δ6-elongase, advantageously in combination with further genes of lipid or fatty acid metabolism. Advantageously, only EPA, DPA or DHA or mixtures thereof are synthesized. Since the fatty acids are synthesized in biosynthesis chains, the respective final products are not present as pure substances in the organisms. Small amounts of the precursor compounds are always also present in the final product. These small amounts are less than 20% by weight, advantageously less than 15% by weight, particularly advantageously less than 10% by weight, very particularly advantageously less than 5, 4, 3, 2 or 1% by weight based on the final products EPA, DPA or DHA or mixtures thereof.

To increase the yield in the process according to the invention for the production of oils and/or triglycerides with a polyunsaturated fatty acid, content which is advantageously increased, it is advantageous to increase the amount of starting product for the synthesis of fatty acids. This can be achieved for example by introducing a nucleic acid which encodes a polypeptide with Δ12-desaturase into the organism. This is particularly advantageous in useful plants, such as oil-producing plants such as plants of the *Brassicaceae* family, such as the genus *Brassica*, for example rape; the *Elaeagnaceae* family, such as the genus *Elaeagnus*, for example the genus and species *Olea europaea* or the family *Fabaceae*, such as the genus *Glycine*, for example the genus and species *Glycine max*, which are high in oleic acid. Since these organisms have an only low linoleic acid content (Mikoklajczak et al. (1961) Journal of the American Oil Chemical Society 38: 678 - 681) it is advantageous to use said Δ12-desaturases for producing the starting material linolenic acid from oleic acid. It is also possible in addition for the starting fatty acids to be provided from outside, but this is less preferred for reasons of cost.

Mosses and algae are the only plant systems known to produce considerable amounts of polyunsaturated fatty acids such as arachidonic acid (ARA) and/or eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Mosses comprise PUFAs in membrane lipids, whereas algae, organisms related to algae, and some fungi also accumulate substantial amounts of PUFAs

in the triacylglycerol fraction. Nucleic acid molecules isolated from strains which accumulate PUFAs also in the triacylglycerol fraction are therefore particularly advantageous for the process of the invention and thus for modifying the lipid and PUFA production system in a plant such as a useful plant such as an oil crop plant, for example oilseed rape, canola, flax, hemp, soybean, sunflower, borage. They can therefore advantageously be used in the process of the invention.

Nucleic acids used in the process of the invention are advantageously derived from plants such as algae, for example algae of the family of Prasinophyceae such as from the genera *Heteromastix, Mammella, Mantoniella, Micromonas, Nephroselmis, Ostreococcus, Prasinocladus, Prasinococcus, Pseudoscourfielda, Pycnoocus, Pyramimonas, Scherffelia* or *Tetraselmis* such as the genera and species *Heteromastix longifillis, Mamiella gilva, Mantoiella squamata, Micromonas pusilla, Nephroselmis olivacea, Nephroselmis pyriformis, Neproselmis rotunda, Ostreococcus tauri, Ostreococcus* sp. *Prasinocladus ascus, Prasinocladus lubricus, Pycnococcus provasolii, Pyramimonas amylifera, Pyramimonas disomata, Pyramimonas obovata, Pyramimonas orientalis, Pyramimonas parkae, Pyramimonas spinefera, Pyramimonas* sp., *Tetraselmis apiculta, Tetraselmis carteriaformis, Tetraselmis chui, Tetraselmis convolutae, Tetraselmis desikacharyi, Tetraselmis gracilis, Tetraselmis hazeni, Tetraselmis impellucida, Tetraselmis inconspicua, Tetraselmis levis, Tetraselmis maculata, Tetraselmis marina, Tetraselmis striata, Tetraselmis subcordiformis, Tetraselmis suecica, Tetraselmis tetrabrachia, Tetraselmis tetrathele, Tetraselmis verrucosa, Tetraselmis verrucosa* fo. rubens *or Tetraselmis* sp. or algae from the family *Euglenacease* such as from the genera *Ascoglena, Astasia, Colacium, Cyclidiopsis, Euglena, Euglenopsis, Hyalophacus, Khawkinea, Lepocinclis, Phacus, Strombomonas* or *Trachelomonas* such as the genera and species *Euglena acus, Euglena geniculata, Euglena gracilis, Euglena mixocylindracea, Euglena rostrifera, Euglena viridis, Colacium stentorium, Trachelomonas cylindrica* or *Trachelomonas volvocina*.

Further advantageous plants are algae such as *Isochrysis* or *Crypthecodinium*, algae/diatoms such as *Thalassiosira* or *Phaeodactylum*, mosses such as *Physcomitrella* or *Ceratodon* or higher plants such as the *Primulaceae* such as *Aleuritia, Calendula stella, Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi such as *Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor* or *Mortierella*, bacteria such as shewanella, yeasts or animals such as nematodes such as *Caenorhabditis*, insects, frogs, sea cucumbers or fishes. The nucleic acid sequences isolated according to the invention are

advantageously derived from an animal from the order of vertebrates. The nucleic acid sequences are preferably derived from the class of *Vertebrata; Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae* or *Oncorhynchus* or *Vertebrata, Amphibia, Anura, Pipidae, Xenopus* or *Evertebrata* such as *Protochordata, Tunicata, Holothuroidea, Cionidae* such as *Amaroucium constellatum, Botryllus schlosseri, Ciona intestinalis, Molgula citrina, Molgula manhattensis, Perophora viridis* or *Styela partita*. The nucleic acids are particularly advantageously derived from fungi, animals or from plants such as algae or mosses, preferably from the order of *Salmoniformes* such as of the family of *Salmonidae* such as of the genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss*, *Trutta trutta* or *Salmo trutta fario*, from algae such as the genera *Mantoniella* or *Ostreococcus* or from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum* or from algae such as *Crypthecodinium*.

In a preferred embodiment, the process further comprises the step of obtaining a cell or a whole plant which comprises the nucleic acid sequences which are used in the process and which code for a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase and, if appropriate, nucleic acid sequences which code for an ω3-desaturase and/or a Δ4-desaturase, it being possible for the cell and/or the useful plant also to comprise further nucleic acid sequences of lipid or fatty acid metabolism. The nucleic acid sequences preferably used in the process are for expression advantageously incorporated into at least one gene construct and/or a vector as described hereinafter, alone or in combination with further nucleic acid sequences which code for proteins of fatty acid or lipid metabolism, and finally transformed into the cell or plant. In a further preferred embodiment, this process further comprises the step of obtaining the oils, lipids or free fatty acids from the useful plants, The cell produced in this way or the useful plant produced in this way is advantageously a cell of an oil-producing plant, vegetable plant, lettuce plant, or ornamental plant or the plant itself as stated above.

Growing means for the cultivation in the case of plant cells, tissue or organs on or in a nutrient medium or of the whole plant on or in a substrate, for example in hydroculture, flower pot soil or on an arable field.

For the purposes of the invention, "transgenic" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (= gene construct) or a vector

comprising the nucleic acid sequences used in the process according to the invention or a plant transformed with the nucleic acid sequences, expression cassette or vector used in the process according to the invention, all those constructions brought about by recombinant methods in which either

a) the nucleic acid sequence, or

b) a genetic control sequence which is operably linked with the nucleic acid sequence, for example a promoter, or

c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to be, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment means the natural genomic or chromosomal locus in the original organism or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, very especially preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequence used in the process according to the invention with the nucleic acid sequence which encodes proteins with corresponding Δ6-desaturase, Δ6-elongase, Δ5-desaturase and Δ5-elongase activity, advantageously in combination with nucleic acid sequences which encode proteins having ω3-desaturase and/or Δ4-desaturase activity – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

A "transgenic plant" for the purposes of the invention is understood as mentined above as meaning that the nucleic acids used in the process are not at their natural locus in the genome of the plant. In this case, it is possible for the nucleic acid sequences to be expressed homologously or heterologously. However, transgenic also means that, while the nucleic acids according to the invention are at their natural position in the genome of the plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences

have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids used in the process according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acid sequences takes place.

Preferred transgenic organisms are useful plants such as oil-producing plants, vegetable plants, lettuce plants or ornamental plants which are advantageously selected from the group of plant families consisting of the families of *Aceraceae, Actinidiaceae, Anacardiaceae, Apiaceae, Arecaceae, Asteraceae, Arecaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Cannaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalidaceae, Papaveraceae, Poaceae, Polygonaceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Scrophulariaceae, Solanaceae, Sterculiaceae* and *Valerianaceae*.

Host plants which are suitable for the nucleic acids, the expression cassette or the vector used in the process according to the invention are, in principle, advantageously all useful plants which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and which are suitable for the expression of recombinant genes. Examples which should be mentioned at this point are plants such as *Arabidopsis, Asteraceae* such as *Calendula* or useful plants such as soybean, peanut, castor-oil plant, sunflower, maize, cotton, flax, oilseed rape, coconut, oil palm, safflower (*Carthamus tinctorius*) or cacao bean. Further advantageous plants are mentioned at other points in this application.

Microorganisms are generally used as intermediate hosts for the production of transgenic useful plants. Such utilizable intermediate host cells are detailed in: Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990).

Expression strains which can advantageously be used for this purpose are, for example, those with a lower protease activity. They are described, for example, in: Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128.

Transgenic plants which comprise the polyunsaturated, long-chain fatty acids synthesized in the process according to the invention can advantageously be marketed directly without there being any need for the oils, lipids or fatty acids synthesized to be isolated. This form of marketing is particularly advantageous.

"Plants" for the purposes of the present invention are intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue.

The compounds produced in the process of the invention can, however, also be isolated from the plants in the form of their oils, fat, lipids and/or free fatty acids. Polyunsaturated fatty acids produced by the process of the invention can be obtained by harvesting the plants or plant cells either from the culture in which they grow or from the field. This can take place by pressing or extracting the plant parts, preferably the plant seeds. It is possible in this connection for the oils, fats, lipids and/or free fatty acids to be obtained by pressing by so-called cold drawing or cold pressing without input of heat. To make it easier to break open the plant parts, specifically the seeds, they are previously crushed, steamed or roasted. The seeds pretreated in this way can then be pressed or extracted with solvent such as warm hexane. The solvent is then removed again. It is possible in this way to isolate more than 96% of the compounds produced in the process of the invention. The products obtained in this way are then processed further, that is to say refined. This entails initially for example the plant mucilage and suspended matter being removed. So-called desliming can take place enzymatically or, for example, chemically/physically by adding acid such as phosphoric acid. The free fatty acids are then removed by treatment with a base, for example sodium hydroxide solution. The resulting product is thoroughly washed with water to remove the alkali remaining in the product, and is dried. In order to remove the coloring matters still present in the product, the products are subjected to a bleaching with, for example, bleaching earth or activated carbon. Finally, the product is also deodorized for example with steam.

The PUFAs or LCPUFAs produced by this process are preferably C_{20} and/or C_{22} fatty acid molecules having at least four double bonds in the fatty acid molecule, preferably five or six

double bonds. These C_{20} and/or C_{22} fatty acid molecules can be isolated from the plant in the form of an oil, lipid or a free fatty acid. Suitable transgenic plants are for example those mentioned above.

These oils, lipids or fatty acids of the invention comprise, as described above, advantageously 6 to 15% palmitic acid, 1 to 6% stearic acid; 7 - 85% oleic acid; 0.5 to 8% vaccenic acid, 0.1 to 1% arachic acid, 7 to 25% saturated fatty acids, 8 to 85% monounsaturated fatty acids and 60 to 85% polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the plants.

Advantageous polyunsaturated, long-chain fatty acids present in the fatty acid esters or fatty acid mixtures such as phosphatidyl fatty acid esters or triacylglyceride esters are preferably at least 10; 11; 12; 13; 14; 15; 16; 17; 18; 19 or 20% by weight based on the total fatty acid content of eicosapentaenoic acid, based on the total fatty acid content, and/or at least 1; 2; 3; 4; 5 or 6% by weight of docosapentaenoic acid, based on the total fatty acid content, and/or at least 1; 2; 3; preferably at least 4; 5; 6; particularly preferably at least 7 or 8 and most preferably at least 9 or 10% by weight of docosahexaenoic acid, based on the total fatty acid content.

The fatty acid esters or fatty acid mixtures which have been produced by the process of the invention further comprise fatty acids selected from the group of fatty acids erucic acid (13-docosaic acid), sterculic acid (9,10-methylene octadec-9-enonic acid), malvalic acid (8,9-methylene heptadec-8-enonic acid), chaulmoogrinic acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienonoic acid), vernonoic acid (9,10-epoxyoctadec-12-enonoic acid), tarinic acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynonic acid, pyrulic acid (t10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynonic acid, petroselenic acid (cis-6-octadecenonic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid, catalpic acid (9t11t13c-octadecatrienoic acid), eleosteric acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienonic acid). In general, the aforementioned fatty acids are

advantageously present only in traces in the fatty acid esters or fatty acid mixtures produced by the process of the invention, meaning that their occurrence, based on the total fatty acid content, is less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, particularly preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very particularly preferably less than 4%, 3%, 2% or 1%. In a further preferred form of the invention the occurrence of these aforementioned fatty acids, based on the total fatty acids, is less than 0.9%; 0.8%; 0.7%; 0.6% or 0.5%, particularly preferably less than 0.4%; 0.3%; 0.2%; 0.1%. The fatty acid esters or fatty acid mixtures produced by the process of the invention advantageously comprise less than 0.1% based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, $C23:6^{\Delta3,8,12,15,18,21}$).

A further embodiment according to the invention is the use of the oils, the lipids, the fatty acids and/or the fatty acid composition, which are produced by the process of the invention, in feeding stuffs, foodstuffs, cosmetics or pharmaceuticals. The oils, lipids, fatty acids or fatty acid mixtures obtained in the process according to the invention can be used for admixture with other oils, lipids, fatty acids or fatty acid mixtures of animal origin, such as, for example, fish oils, in the manner with which the skilled worker is familiar. These oils, lipids, fatty acids or fatty acid mixtures which are produced in this way and consist of vegetable and animal components can also be used for the preparation of feeding stuffs, foodstuffs, cosmetics or pharmaceuticals.

The term "oil", "lipid" or "fat" is understood as meaning a fatty acid mixture comprising unsaturated and/or saturated, preferably esterified fatty acid(s). It is preferred that the oil, fat or lipid is high in polyunsaturated free or advantageously esterified fatty acid(s), in particular linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid. Preferably, the amount of unsaturated esterified fatty acids is approximately 30%, with an amount of 50% being especially preferred and an amount of 60%, 70%, 80% or more being most preferred. The amount of the fatty acid can be determined by gas chromatography after converting the fatty acids into the methyl esters by transesterification. The oil, lipid or fat can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and the like. In particular, the amount of the various fatty acids can vary, depending on the starting plant.

As described above, the polyunsaturated fatty acid esters advantageously having three, four, five or six, particularly advantageously having five or six double bonds and which have been prepared in the process advantageously take the form of fatty acid esters, for example, sphingolipid esters, phosphoglyceride esters, lipid esters, glycolipid esters, phospholipid esters, monoacylglycerol esters, diacylglycerol esters, triacylglycerol esters or other fatty acid esters, preference being given to phospholipid esters and/or triacylglycerol esters.

Starting with the polyunsaturated fatty acid esters produced thus in the process according to the invention and advantageously having at least three, four, five or six double bonds, the polyunsaturated fatty acids which are present can be liberated for example via treatment with alkali, for example with aqueous KOH or NaOH, or by acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification with, for example, H_2SO_4. However, the fatty acids can also be liberated directly without the above-described processing steps.

Substrates of the nucleic acid sequences used in the process which encode polypeptides with Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase activity and optionally nucleic acid sequences which encode polypetides having ω3-desaturase and/or Δ4-desaturase activity, and/or of the further nucleic acids which are used, such as the nucleic acid sequences which encode polypeptides of the fatty acid or lipid metabolism selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously C_{16}-, C_{18}- or C_{20}-fatty acids. Preferably, the fatty acids converted in the process as substrates are converted in the form of their acyl-CoA esters and/or in the form of their phospholipid esters.

To produce the long-chain PUFAs according to the invention, the saturated, monounsaturated C_{16}-fatty acids and/or polyunsaturated C_{18}-fatty acids must first, depending on the substrate, be desaturated and/or elongated or only deaturated by the enzymatic activity of a desaturase and/or elongase and subsequently elongated by at least two carbon atoms by an elongase. After one elongation cycle, this enzyme activity leads either starting from C_{16}-fatty acids to C_{18}-fatty acids

or starting from C_{18}-fatty acids to C_{20}-fatty acids, and after two elongation cycles starting from C_{16}-fatty acids leads to C_{20}-fatty acids. The activity of the desaturases or elongases used in the process according to the invention preferably leads to C_{20}- and/or C_{22}-fatty acids, advantageously with at least two or three double bonds in the fatty acid molecule, preferably with four, five or six double bonds, especially preferably to C_{22}-fatty acids with at least five double bonds in the fatty acid molecule. Especially preferred products of the process according to the invention are eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid. The C_{18}-fatty acids with at least two double bonds in the fatty acid can be elongated by the enzymatic activity according to the invention in the form of the free fatty acid or in the form of the esters, such as phospholipids, glycolipids, sphingolipids, phosphoglycerides, monoacylglycerol, diacylglycerol or triacylglycerol.

The preferred biosynthesis site of fatty acids, oils, lipids or fats in the plants which are advantageously used is, for example, generally the seed or cell layers of the seed, so that seed-specific expression of the nucleic acids used in the process makes sense. However, it is obvious that the biosynthesis of fatty acids, oils or lipids need not be limited to the seed tissue, but may also take place in a tissue specific manner in all of the remaining parts of the plant, for example in epidermal cells or in the tubers. The synthesis advantageously takes place according to the inventive process in the vegetative (somatic) tissue.

Owing to the method according to the invention, the polyunsaturated fatty acids which are produced can, in principle, be increased in two ways in the plants used in the process. Advantageously the pool of free polyunsaturated fatty acids and/or the amount of the esterified polyunsaturated fatty acids produced by the process can be increased. Advantageously, the pool of esterified polyunsaturated fatty acids in the transgenic plants is increased by the process according to the invention, advantageously in the form of the phosphatidyl esters and/or triacyl esters.

The sequences used in the process of the invention are cloned singly into expression constructs or provided on a joint recombinant nucleic acid molecule and used for introduction and for expression in organisms. These expression constructs make it possible for the polyunsaturated fatty acids produced in the process of the invention to be synthesized optimally.

The nucleic acids used in the process may, after introduction into a plant or plant cell, either be located on a separate plasmid or advantageously be integrated into the genome of the host cell. In the case of integration into the genome, the integration may be random or take place by recombination such that the native gene is replaced by the introduced copy, thus modulating production of the desired compound by the cell, or through use of a gene in trans, so that the gene is functionally connected to a functional expression unit which comprises at least one sequence ensuring the expression of a gene and at least one sequence ensuring the polyadenylation of a functionally transcribed gene. The nucleic acid sequences are advantageously introduced into the plants via multiexpression cassettes or constructs for multiparallel expression, i.e. the nucleic acid sequences are present in a joint expression unit.

The nucleic acid construct may comprise more than one nucleic acid sequence coding for a polypeptide having the enzymatic activity of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase, and/or ω3-desaturase. It is also possible for a plurality of copies of a nucleic acid sequence coding for a polypeptide having the enzymatic activity of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase, and/or ω3-desaturase to be present.

For the introduction, the nucleic acids used in the process are advantageously amplified and ligated in the known manner. Preferably, a procedure following the protocol for Pfu DNA polymerase or a Pfu/Taq DNA polymerase mixture is followed. The primers are selected depending on the sequence to be amplified. The primers should expediently be chosen in such a way that the amplicon comprises the entire codogenic sequence from the start codon to the stop codon. After the amplification, the amplificon is expediently analyzed. For example, the analysis can be carried out by gel-electrophoretic separation with respect to quality and quantity. Thereafter, the amplicon can be purified following a standard protol (for example Qiagen). An aliquot of the purified amplicon is then available for the subsequent cloning step. Suitable cloning vectors are generally known to the skilled worker. These include, in particular, vectors which are capable of replication in microbial systems, that is to say mainly vectors which ensure efficient cloning in yeasts or fungi and which make possible the stable transformation of plants. Those which must be mentioned in particular are various binary and cointegrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule,

characterized in that they comprise at least the vir genes required for the Agrobacterium-mediated transformation and the T-DNA-delimiting sequences (T-DNA border). These vector systems preferably also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers, by means of which suitably transformed organisms can be identified. While in the case of cointegrated vector systems vir genes and T-DNA sequences are arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir genes. Owing to this fact, the last-mentioned vectors are relatively small, easy to manipulate and capable of replication both in E. coli and in Agrobacterium. These binary vectors include vectors from the series pBIB-HYG, pPZP, pBecks, pGreen. In accordance with the invention, pBin19, pBI101, pBinAR, pGPTV and pCAMBIA are used by preference. An overview of the binary vectors and their use is found in Hellens et al. (2000) Trends in Plant Science 5: 446–451.

In order to prepare the vectors, the vectors can first be linearized with restriction endonuclease(s) and then modified enzymatically in a suitable manner. Thereafter, the vector is purified, and an aliquot is employed for the cloning step. In the cloning step, the enzymatically cleaved and, if appropriate, purified amplificate is cloned with vector fragments which have been prepared in a similar manner, using ligase. In this context, a particular nucleic acid construct, or vector or plasmid construct, can have one or more than one codogenic gene segments. The codogenic gene segments in these constructs are preferably linked operably with regulatory sequences. The regulatory sequences include, in particular, plant sequences such as the above-described promoters and terminators. The constructs can advantageously be stably propagated in microorganisms, in particular Escherichia coli and Agrobacterium tumefaciens, under selective conditions and thus make possible the transfer of heterologous DNA into plants.

The nucleic acid sequences and nucleic acid constructs used in the inventive process can be introduced into microorganisms and then into plants, advantageously using cloning vectors, and thus be used in the transformation of plants such as those which are published in and cited therein: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, p. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev.

Plant Physiol. Plant Molec. Biol. (1991) 42: 205-225. Thus, the nucleic acids, nucleic acid constructs and/or vectors used in the process can be used for the recombinant modification of a broad spectrum of plants so that the latter become better and/or more efficient LCPUFA producers.

Owing to the introduction of a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and Δ5-elongase gene into a plant, alone or in combination with other genes, it is not only possible to increase biosynthesis flux towards the end product, but also to increase, or to create *de novo* the corresponding triacylglycerol and/or phosphatidylester composition. Likewise, the number or activity of other genes which are involved in the import of nutrients which are required for the biosynthesis of one or more fatty acids, oils, polar and/or neutral lipids, can be increased, so that the concentration of these precursors, cofactors or intermediates within the cells or within the storage compartment is increased, whereby the ability of the cells to produce PUFAs, as described below, is enhanced further. By optimizing the activity or increasing the number of one or more of the Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase genes which are involved in the biosynthesis of these compounds, or by destroying the activity of one or more genes which are involved in the degradation of these compounds, it may be possible to increase the yield, production and/or production efficiency in fatty acid and lipid molecules from organisms and advantageously from plants.

The nucleic acid molecules used in the process of the invention code for proteins or parts thereof, whereas the proteins or the individual protein or parts thereof comprises an amino acid sequence which has sufficient homology to an amino acid sequence which is depicted in the sequences SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172 or SEQ ID NO: 52 and, if appropriate, SEQ ID NO: 194 or SEQ ID NO: 78, so that the proteins or parts thereof still have a Δ6-desaturase, Δ6-elongase, Δ5-desaturase and/or Δ5-elongase activity and, if appropriate, a Δ4-desaturase and/or ω3-desaturase activity. The proteins or parts thereof which is/are encoded by the nucleic acid molecule/nucleic acid molecules preferably still have its/their essential enzymatic activity and the ability to participate in the metabolism of compounds necessary for constructing cell membranes or lipid bodies in organisms, advantageously in plants, or in the transport of molecules across these membranes. The proteins encoded by the nucleic acid molecules are at least about 60% and preferably at least about 70%, 80% or 90%, and particularly preferably at

least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequences depicted in SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78. Homology or homologous means in the context of the invention identity or identical.

The homology was calculated over the entire amino acid or nucleic acid sequence region. To compare various sequences, the skilled worker has available a series of programs which are based on various algorithms. The algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution (1987) 25: 351-360; Higgins et al. (1989) CABIOS 5: 151–153) or the programs Gap and BestFit (Needleman and Wunsch (1970) J. Mol. Biol. 48: 443-453 and Smith and Waterman (1981) Adv. Appl. Math. 2: 482-489), which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used to carry out the sequence comparisons. The sequence homology data given above in % were determined over the entire sequence region using the program GAP with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for sequence comparisons.

Essential enzymatic activity of the ω3-desaturase, Δ6-desaturase, Δ6-elongase, Δ5-elongase, Δ4-desaturase and/or Δ5-desaturase used in the process of the invention means that, compared with the proteins/enzymes encoded by the sequence having SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, they still have an enzymatic activity of at least 10%, preferably of at least 20%, particularly preferably of at least 30% and most preferably of at least 40, 50 or 60%, and thus are able to participate in the metabolism of compounds necessary for synthesizing fatty acids, advantageously fatty acid esters such as phosphatidyl esters and/or triacylglyceride esters, in a plant or plant cell, or in the transport of molecules across membranes.

Nucleic acids which can be advantageously used in the process are derived from bacteria, fungi, diatoms, animals such as *Caernorhabditis* or *Oncorhynchus* or plants such as algae or mosses such as the genera *Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Pytium irregulare, Mantoniella, Ostreococcus, Isochrysis, Aleurita, muscarioides, Mortierella, Borago, Phaeodactylum, Crypthecodinium*, specifically from the genera and species

Pytium irregulare, Oncorhynchus mykiss, Xenopus laevis, Ciona intestinalis, Thalassiosira pseudonona, Mantoniella squamata, Ostreococcus sp., *Ostreococcus tauri, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium* sp., *Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricornutum, Caenorhabditis elegans* or particularly advantageously from *Pytium irregulare, Thraustochytrium* sp. and/or *Ostreococcus tauri*.

It is possible additionally to use in the process of the invention nucleotide sequences which code for a Δ12-desaturase, Δ9-elongase or Δ8-desaturase. The nucleic acid sequences used in the process are advantageously introduced in an expression cassette which makes expression of the nucleic acids in plants possible.

The nucleic acid sequences which code for the Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase are functionally linked to one or more regulatory signals to increase the gene expression. These regulatory sequences are intended to make targeted expression of the genes possible. This may mean for example, depending on the plant, that the gene is expressed and/or overexpressed only after induction, or that it is expressed and/or overexpressed immediately. Sequences advantageously used for the expression make constitutive expression possible, such as CaMV35S, CaMV36S, CaMV35Smas, nos, mas, ubi, stpt, lea or Super promoter. Expression preferably takes place in vegetative tissue as described above. In another preferred embodiment, the expression takes place in seeds.

These regulatory sequences are for example sequences to which inducers or repressors bind and thus regulate the expression of the nucleic acid. In addition to the regulatory sequences which are not linked in their natural locus to the nucleic acid sequences, or instead of these sequences, the natural regulation of these sequences may still be present before the actual structural genes and, if appropriate, have been genetically modified so that natural regulation is switched off and expression of the genes is increased. The gene construct may additionally advantageously also comprise one or more so-called "enhancer sequences" functionally linked to the promoter, which make increased expression of the nucleic acid sequence possible. Additional advantageous sequences can also be inserted at the 3' end of the DNA sequences, such as further regulatory

elements or terminators. Advantageous terminators are for example viral terminators such as the 35S terminator or others. The nucleic acid sequences used in the process according to the invention may be present in one or more copies of the expression cassette (= gene construct). Preferably, only one copy of the genes is present in each expression cassette. This gene construct, or the gene constructs, can be introduced into the plant simultaneously or successively and expressed together in the host organism. In this context, the gene construct(s) can be inserted in one or more vectors and be present in the cell in free form, or else be inserted in the genome. It is advantageous for the insertion of further genes in the plant when the genes to be expressed are present together in one gene construct. However, it is also possible to introduce in each case one gene construct containing a nucleic acid sequence into a plant and to cross the resulting plants with one another in order to obtain progeny which contains all gene contructs together.

In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the gene expression of the genes introduced, thus enhancing it. Thus, an enhancement of the regulatory elements, advantageously at the transcriptional level, may take place by using strong transcription signals such as promoters and/or enhancers. In addition, however, enhanced translation is also possible, for example by improving the stability of the mRNA.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations, each of the nucleic acids which encode Δ6-desaturase, Δ6-elongase, Δ5-desaturase or Δ5-elongase and if appropriate the ω3-desaturase or Δ4-desaturase and which are used in the process should be expressed under the control of a separate promoter. This can be identical or different for each of the sequences. In this context, the expression cassette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site for insertion of the nucleic acid to be expressed, which cleavage site is advantageously in a polylinker. If appropriate, a terminator can be positioned behind the polylinker. This sequence is repeated several times, preferably three, four, five or six times, so that up to six genes can be combined in one construct and thus introduced into the transgenic plant in order to be expressed. To express the nucleic acid sequences, the latter are inserted behind the promoter via the suitable cleavage site, for example in the polylinker. Advantageously, each nucleic acid sequence has its own promoter and, if appropriate, its own terminator. However, it is also possible to insert a plurality of nucleic acid sequences behind a promoter and, if appropriate, before a terminator.

Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to say a nucleic acid sequence can be inserted at the first or last position in the cassette without the expression being substantially influenced by the position. In an advantageous embodiment, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminators can be used in the expression cassette. In a further advantageous embodiment, identical promoters such as the CaMV35S promoter can also be used.

As described above, the transcription of the genes which have been introduced should advantageously be terminated by suitable terminators at the 3' end of the biosynthesis genes which have been introduced (behind the stop codon). An example of a sequence which can be used in this context is the OCS 1 or the 35SCaMV terminator. As is the case with the promoters, different terminator sequences should be used here for each gene.

As described above, the gene construct can also comprise further genes to be introduced into the organisms. It is possible and advantageous to introduce into the host plants, and to express therein, regulatory genes such as genes for inductors, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a biosynthesis pathway. These genes can be of heterologous or of homologous origin. Moreover, further biosynthesis genes of the fatty acid or lipid metabolism can advantageously be present in the nucleic acid construct, or gene construct or alternatively, these genes can also be present on one further or more further nucleic acid constructs. A biosynthesis gene of the fatty acid or lipid metabolism which is preferably chosen is one or more genes selected from the group of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl- transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) or combinations thereof. Especially advantageous nucleic acid sequences are biosynthesis genes of the fatty acid or lipid metabolism selected from the group of the acyl-CoA:lysophospholipid acyltransferase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase and/or Δ9-elongase.

In this context, the abovementioned nucleic acids or genes can be cloned into expression cassettes, like those mentioned above, in combination with other elongases and desaturases and used for transforming plants with the aid of Agrobacterium.

The term "vector" used in this description relates to a nucleic acid molecule which is capable of transporting another nucleic acid to which it is bound. One type of vector is a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated. A further type of vector is a viral vector, it being possible for additional DNA segments to be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they have been introduced (for example bacterial vectors with bacterial replication origin). Other vectors are advantageously integrated into the genome of a host cell when they are introduced into the host cell, and thus replicate together with the host genome. Moreover, certain vectors can govern the expression of genes with which they are in operable linkage. These vectors are referred to in the present context as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids. In the present description, "plasmid" and "vector" can be used exchangeably since the plasmid is the form of vector which is most frequently used. However, the invention is also intended to cover other forms of expression vectors, such as viral vectors, which exert similar functions. Furthermore, the term vector is also intended to encompass other vectors with which the skilled worker is familiar, such as phages, viruses such as SV40, CMV, TMV, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA.

The recombinant expression vectors advantageously used in the process comprise the nucleic acid sequences or the above-described gene construct used in the process in a form which is suitable for expressing the nucleic acids used in a host cell, which means that the recombinant expression vectors comprise one or more regulatory sequences, which are selected on the basis of the host cells to be used for the expression, which regulatory sequence(s) is/are linked operably with the nucleic acid sequence to be expressed. In a recombinant expression vector, "linked operably" means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the expression of the nucleotide sequence is made possible and they are bound to each other in such a way that both sequences carry out the predicted function which is ascribed to the sequence (for example in an in-vitro transcription/translation system, or in a host cell if the vector is introduced into the host cell). The term "regulatory sequence" is

intended to comprise promoters, enhancers and other expression control elements (for example polyadenylation signals). These regulatory sequences are described, for example, in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Florida, Ed.: Glick and Thompson, Chapter 7, 89-108, including the references cited therein. Regulatory sequences comprise those which govern the constitutive expression of a nucleotide sequence in many types of host cell and those which govern the direct expression of the nucleotide sequence only in specific host cells under specific conditions. The skilled worker knows that the design of the expression vector can depend on factors such as the choice of host cell to be transformed, the desired degree of expression of the protein and the like.

The recombinant expression vectors used can be designed for the expression of the nucleic acid sequences used in the process in such a way that they can be transformed into prokaryotic intermediate hosts and finally, after introduction into the plants, make expression of the genes possible therein. This is advantageous because on account of simplicity, intermediate steps in vector construction are frequently carried out in microorganisms. For example, the Δ6-desaturates, Δ6-elongase, Δ5-desaturate and/or Δ5-elongase genes can be expressed in bacterial cells, insect cells (using baculovirus expression vectors), yeast cells and other fungal cells (see Romanos, M.A., et al. (1992) Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Editors, pp. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J., & Punt, P.J. (1992) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Editors, pp. 1-28, Cambridge University Press: Cambridge), Algae (Falciatore et al. (1999) Marine Biotechnology.1: (3):239-251), ciliates, with vectors following a transformation process as described in WO 98/01572, and preferably in cells of multicellular plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" Plant Cell Rep.:538-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter 6/7, pp. 71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer in: Transgenic Plants, vol. 1, Engineering and Utilization, Editors.: Kung and R. Wu, Academic Press (1993), 128-43;

Potrykus (1991) Annu. Rev. Plant Physiol. Plant Molec. Biol. 42: 205-225 (and references cited therein)). Suitable hosts are what are further discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). The recombinant expression vector may alternatively be transcribed and translated in vitro for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes usually takes place with vectors which comprise constitutive or inducible promoters which control the expression of fusion or non-fusion proteins. Typical fusion expression vectors are inter alia pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), of which glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused to the recombinant target protein.

Examples of suitable inducible non-fusion E. coli expression vectors are inter alia pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression by the pTrc vector is based on transcription by host RNA polymerase from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector is based on transcription from a T7-gn10-lac fusion promoter which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ prophage which harbors a T7 gn1 gene under transcription control of the lacUV 5 promoter.

Other vectors suitable in prokaryotic organisms are known to the skilled worker; these vectors are for example in *E. coli* pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCI, in *streptomyces* pIJ101, pIJ364, pIJ702 or pIJ361, in *bacillus* pUB110, pC194 or pBD214, in *corynebacterium* pSA77 or pAJ667.

In a further embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in the yeast *S. cerevisiae* include pYeDesaturasec1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and

processes for constructing vectors suitable for use in other fungi, such as the filamentous fungi, are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., editors, pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi (J.W. Bennet & L.L. Lasure, Editors, pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are for example pAG-1, YEp6, YEp13 or pEMBLYe23.

Alternatively, the nucleic acid sequences used in the process of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expressing proteins in cultured insect cells (e.g. Sf9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The above mentioned vectors provide only a small survey of possible suitable vectors. Further plasmids are known to the skilled worker and are described for example in: Cloning Vectors (Editors Pouwels, P.H. et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Further suitable expression systems for prokaryotic and eukaryotic cells see in chapters 16 and 17 of Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

The genes used in the process can also be expressed in single-celled plant cells (such as algae), see Falciatore et al. (1999) Marine Biotechnology 1 (3):239-251 and references cited therein, and in plant cells from higher plants (for example spermatophytes such as arable crops). Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) Plant Mol. Biol. 20:1195-1197; and Bevan, M.W. (1984) Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38.

A plant expression cassette preferably comprises regulatory sequences which are capable of governing the expression of genes in plant cells and are linked operably so that each sequence can fulfil its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as gene 3 of the Ti plasmid pTiACH5 (Gielen et al., (1984) EMBO J. 3 835 et

seq.), which is known as octopine synthase, or functional equivalents thereof, but all other terminators which are functionally active in plants are also suitable.

Since the regulation of plant gene expression is very often not limited to the transcriptional level, a plant expression cassette preferably comprises other sequences which are linked operably, such as translation enhancers, for example the overdrive sequence, which enhances the tobacco mosaic virus 5' - untranslated leader sequence, which increases the protein/RNA ratio (Gallie et al. (1987) Nucl. Acids Research 15:8693-8711).

As described above, the plant gene expression must be linked operably with a suitable promoter which controls gene expression. Advantageously utilizable promoters are constitutive promoters (Benfey et al., EMBO J. (1989) 8: 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al. (1980) Cell 21: 285-294), 19S CaMV (see also US 5352605 and WO 84/02913), or plant promoters, such as the promoter of the Rubisco small subunit, which is described in US 4,962,028.

Other preferred sequences for use for functional connection in plant gene expression cassettes are targeting sequences which are necessary for guiding the gene product into its appropriate cellular compartment, for example into the vacuoles, the cell nucleus, all types of plastids such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells; (see a review in Kermode (1996) Crit. Rev. Plant Sci. 15(4): 284-423 and literature cited therein).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via traditional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction as used in the present context are intended to encompass the multiplicity of prior-art methods for introducing heterologous nucleic acids (for example DNA) into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals such as Methods in Molecular Biology,

1995, Vol. 44, *Agrobacterium* protocols, Ed: Gartland and Davey, Humana Press, Totowa, New Jersey.

The term "nucleic acid (molecule)" as used herein comprises in an advantageous embodiment additionally the untranslated sequence located at the 3' end and at the 5' end of the coding gene region: at least 500, preferably 200, particularly preferably 100 nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, particularly preferably 20 nucleotides of the sequence downstream of the 3' end of the coding gene region. An "isolated" nucleic acid molecule is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably has no sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid is derived (e.g. sequences located at the 5' and 3' ends of the nucleic acid). In various embodiments, the isolated Δ6-desaturase, Δ6-elongase or Δ5-desaturase and, if appropriate, the ω3-desaturase or Δ4-desaturase molecule used in the process may for example comprise less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived.

The nucleic acid molecules used in the process can be isolated by using standard techniques of molecular biology and the sequence information provided herein. It is also possible for example with the aid of comparative algorithms to identify a homologous sequence or homologous, conserved sequence regions at the DNA or amino acid level. These can be used as hybridization probe in standard hybridization techniques (as described for example in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for isolating further nucleic acid sequences useful in the process. The nucleic acid molecule used in the process, or parts thereof, can moreover be isolated by polymerase chain reaction, in which case oligonucleotide primers based on this sequence or on parts thereof are used (e.g. a nucleic acid molecule comprising the complete sequence or a part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been constructed on the basis of this identical sequence). For example, mRNA can be isolated from cells (e.g. by the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA can be

prepared with the aid of reverse transcriptase (e.g. Moloney MLV reverse transcriptase obtainable from Gibco/BRL, Bethesda, MD or AMV reverse transcriptase, obtainable from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for amplification by means of polymerase chain reaction can be constructed on the basis of one of the sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 or with the aid of the amino acid sequences depicted in SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78. A nucleic acid of the invention can be amplified by standard PCR amplification techniques using cDNA or alternatively genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified in this way can be cloned into a suitable vector and characterized by DNA sequence analysis. Oligonucleotides can be prepared by standard synthetic methods, for example using an automatic DNA synthesizer.

Homologs of the Δ5-elongase, ω3-desaturase, Δ6-desaturase, Δ6-elongase, Δ4-desaturase or Δ5-desaturase nucleic acid sequences used, having the sequence SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, mean for example allelic variants having at least about 40, 50 or 60%, preferably at least about 60 or 70%, more preferably at least about 70 or 80%, 90% or 95% and even more preferably at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity or homology to one of the nucleotide sequences shown in SEQ ID NO: 64, 66, 68 or 70, to one of the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 or 41, to one of the nucleotide sequences shown in SEQ ID NO: 171, 173, 175, 177, 179, 181 or 183, to one of the nucleotide sequences shown in SEQ ID NO: 51, 53 or 55, to one of the nucleotide sequences shown in SEQ ID NO: 193 or 195 or to one of the nucleotide sequences shown in or SEQ ID NO: 77, 79, 81, 83, 85, 87, 89, 91 or 93, especially the nucleotide sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, or their homologs, derivatives or analogs or parts thereof. Also included are isolated nucleic acid molecules of a nucleotide sequence which hybridize for example under stringent conditions to one of the nucleotide sequences shown in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 or a part thereof. A part means in this connection according to the invention that at least 25 base pairs (= bp), 50 bp, 75 bp, 100 bp, 125 bp or 150 bp, preferably at least 175 bp,

200 bp, 225 bp, 250 bp, 275 bp or 300 bp, particularly preferably 350 bp, 400 bp, 450 bp, 500 bp or more base pairs are used for the hybridization. It is also possible advantageously to use the complete sequence. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from the sequence depicted in SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, but where the enzyme activity of the proteins encoded thereby is substantially retained for the insertion.

Nucleic acid molecules advantageous for the process of the invention can be isolated on the basis of their homology to the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase and/or Δ6-elongase nucleic acid sequences disclosed herein by using the sequences or a part thereof as hybridization probe in standard hybridization techniques under stringent hybridization conditions. It is possible in this connection for example to use isolated nucleic acid molecules which are at least 15 nucleotides long and hybridize under stringent conditions with the nucleic acid molecules which comprise a nucleotide sequence of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77. It is also possible to use nucleic acid molecules having at least 25, 50, 100, 250 or more nucleotides.

The term "hybridizes under stringent conditions" as used herein is intended to describe hybridization and washing conditions under which nucleic acid sequences which are at least 60% mutually homologous normally remain hybridized together. The conditions are preferably such that sequences which are at least about 65%, preferably at least about 70% and particularly preferably at least about 75% or more mutually homologous normally remain hybridized together. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-restrictive example of stringent hybridization conditions are hybridizations in 6 x sodium chloride/sodium citrate (= SSC) at about 45°C, followed by one or more washing steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker is aware that these hybridization conditions differ depending on the type of nucleic acid and, for example organic solvents are present, in relation to the temperature and the concentration of the buffer. The temperature for example under "standard hybridization conditions" is, depending on the type of nucleic acid, between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic

solvent, for example 50% formamide, is present in the abovementioned buffer, the temperature under standard conditions is about 42°C. The hybridization conditions for DNA:DNA hybrids are preferably for example 0.1 x SSC and 20°C to 45°C, preferably 30°C to 45°C. The hybridization conditions for DNA:RNA hybrids are preferably for example 0.1 x SSC and 30°C to 55°C, preferably 45°C to 55°C. The aforementioned hybridization temperatures are determined for example for a nucleic acid with a length of about 100 bp (= base pairs) and a G + C content of 50% in the absence of formamide. The skilled person knows how the necessary hybridization conditions can be determined on the basis of textbooks such as the abovementioned or from the following textbooks Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (editors) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (editor) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

In order to determine the percentage of homology (= identity) of two amino acid sequences (for example one of the sequences of SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78) or of two nucleic acids (for example SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77), the sequences are written one under the other in order to be able to compare them optimally (for example, gaps may be introduced into the sequence of a protein or of a nucleic acid in order to generate optimal alignment with the other protein or the other nucleic acid). Then, the amino acid radicals or nucleotides at the corresponding amino acid positions or nucleotide positions are compared. If a position in a sequence is occupied by the same amino acid radical or the same nucleotide as the corresponding position in another sequence, then the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity"). The percentage of homology between the two sequences is a function of the number of identical positions which the sequences share (i.e. % homology = number of identical positions/total number of positions x 100). The programs and algorithms used to determine the homology are described above.

An isolated nucleic acid molecule which codes for an ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase and/or Δ6-elongase which is used in the process and

which is homologous to a protein sequence of SEQ ID NO: 65, SEQ ID NO: 2, SEQ ID NO: 172, SEQ ID NO: 52, SEQ ID NO: 194 or SEQ ID NO: 78 can be generated by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77, so that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations may be introduced into one of the sequences of SEQ ID NO: 64, SEQ ID NO: 1, SEQ ID NO: 171, SEQ ID NO: 51, SEQ ID NO: 193 or SEQ ID NO: 77 by standard techniques such as site-specific mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are preferably produced at one or more of the predicted nonessential amino acid residues. In a "conservative amino acid substitution" the amino acid residue is replaced by an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid residue in an ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase or Δ6-elongase is thus preferably replaced by another amino acid residue from the same side-chain family. An alternative possibility in another embodiment is to introduce the mutations randomly over the whole or a part of the ω3-desaturase-, Δ6-desaturase-, Δ5-desaturase-, Δ5-elongase-, Δ4-desaturase- or Δ6-elongase-encoding sequence, e.g. by saturation mutagenesis, and the resulting mutants can be screened for the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase, Δ4-desaturase or Δ6-elongase activity described herein in order to identify mutants which have retained the ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ5-elongase,Δ4-desaturase or Δ6-elongase activity. The encoded protein can be recombinantly expressed after the mutagenesis, and the activity of the protein can be determined for example by using the assays described herein.

The invention is illustrated in greater detail by the examples which follow, which are not to be construed as limiting. The content of all of the references, patent applications, patents and

published patent applications cited in the present patent application is herewith incorporated by reference.

The following table shows the sequence identifiers as used in the priority application of February 21, 2006, with the German application number 102006008030.0, and the corresponding sequence identifiers in this subsequent application. The nucleic acid sequence identified by SEQ ID NO: 1 of the priority application corresponds for example to the nucleic acid sequence identified by SEQ ID NO: 64 of the subsequent application.

Table of concordance of sequence identifiers of the priority application and the sequence identifiers in the subsequent application:

SEQ ID NO: Priority application German application number 102006008030.0	**SEQ ID NO: this subsequent application**	**Organism**
1	64	*Ostreococcus tauri*
2	65	*Ostreococcus tauri*
3	1	*Phytium irregulare*
4	2	*Phytium irregulare*
5	171	*Traustochytrium sp.*
6	172	*Traustochytrium sp.*
7	51	*Thraustochytrium ssp.*
8	52	*Thraustochytrium ssp.*
9	193	*Phytophthora infestans*
10	194	*Phytophthora infestans*
11	77	*Traustochytrium sp.*
12	78	*Traustochytrium sp.*
13	109	*Ostreococcus tauri*
n.a.	110	*Ostreococcus tauri*
14	122	*Ostreococcus tauri*
n.a.	123	*Ostreococcus tauri*
15	143	*Ostreococcus tauri*

16	144	*Ostreococcus tauri*
17	161	*Cauliflower mosaic virus*
18	162	*Cauliflower mosaic virus*
19	163	*Thalassiosira pseudonana*
20	164	*Thalassiosira pseudonana*

Examples

Example 1: General cloning methods

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of *Escherichia coli* cells, bacterial cultures and the sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

Example 2: Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced with an ABI laser fluorescence DNA sequencer by the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74: 5463-5467). Fragments resulting from a polymerase chain reaction were sequenced and verified to avoid polymerase errors in constructs to be expressed.

Example 3: Cloning of genes from Ostreococcus tauri

It was possible by searching for conserved regions in an *Ostreococcus tauri* sequence database (genomic sequences) in each case a sequence coding for a protein having Δ5-elongase activity or Δ6-elongase activity. These are the following sequences:

Gene name	SEQ ID	Amino acids
OtELO1.1, (Δ6-Elongase)	SEQ ID NO: 143	292
OtELO2.1, (Δ5-Elongase)	SEQ ID NO: 109	300

OtElo2.1 shows greatest similarity to an elongase from *Danio rerio* (GenBank AAN77156;

approx. 26% identity), whereas OtElo1.1 shows greatest similarity to the elongase from *Physcomitrella* (PSE) (approx. 36% identity) (alignments were carried out with the tBLASTn algorithm (Altschul et al. (1990) J. Mol. Biol. 215: 403-410)).

The cloning of the elongases was carried out as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down and resuspended in 100 µl of double-distilled water and stored at -20°C. The corresponding genomic DNAs were amplified by the PCR method. The corresponding primer pairs were selected so that they harbored the yeast consensus sequence for high-efficiency translation (Kozak (1986) Cell 44: 283-292) beside the start codon. Amplification of the OtElo DNAs was carried out in each case with 1 µl of thawed cells, 200 µM dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 µl. The conditions for the PCR were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes.

Example 4: Optimization of elongase genes from *Ostreococcus tauri*

Elongases from the organism *Ostreococcus tauri* were isolated as described in example 3. In order to achieve an increase in the content of C22 fatty acids, the sequences SEQ ID NO: 143 (Δ6-elongase) and SEQ ID NO: 109 (coding for a protein identified by SEQ ID NO: 110) (Δ5-elongase) were adapted to the codon usage in oilseed rape, flax and soybean. For this purpose, the amino acid sequence of the Δ6-elongase and of the Δ5-elongase (SEQ ID NO: 144 for the Δ6-elongase; SEQ ID NO: 65 for the Δ5-elongase) was back-translated to obtain degenerate DNA sequences. These DNA sequences were adapted by means of the GeneOptimizer program (from Geneart, Regensburg) to the codon usage in oilseed rape, soybean and flax, taking account of the natural frequency of individual codons. The optimized sequences obtained in this way, which are indicated in SEQ ID NO: 64 (Δ5-elongase) and SEQ ID NO: 122 (coding for a protein identified by SEQ ID NO: 123) (Δ6-elongase) were synthesized in vitro.

Example 5: Cloning of expression plasmids for heterologous expression in yeasts

To characterize the function of the optimized nucleic acid sequences, the open reading frames of the respective DNAs were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), resulting in the plasmids pOTE1.2 (comprising the Δ6-

elongase sequence) and pOTE2.2 (comprising the Δ5-elongase sequence).

Overview of the elongase sequences cloned into the yeast vector pYES2.1/V5-His-TOPO:

Gene name	SEQ ID	Amino acids
pOTE1.1, (Δ6-elongase)	SEQ ID NO: 143	292
pOTE1.2, (Δ6-elongase)	SEQ ID NO: 122	292, codon-optimized
pOTE2.1, (Δ5-elongase)	SEQ ID NO: 109	300
pOTE2.2, (Δ5-elongase)	SEQ ID NO: 64	300, codon-optimized

The *Saccharomyces cerevisiae* strain 334 was transformed by electroporation (1500 V) with the vectors pOTE1.2 and pOTE2.2 and with the comparative constructs pOTE1.1 and pOTE2.1 which comprise the natural nucleic acid sequence coding for the Δ6-elongase and Δ5-elongase, respectively. A yeast transformed with the empty vector pYES2 was used as control. The transformed yeasts were selected on complete minimal medium (CMdum) agar plates with 2% glucose but without uracil. After the selection, three transformants in each case were selected for further functional expression.

To express the Ot elongases, initially precultures composed of in each case 5 ml of CMdum liquid medium with 2% (w/v) raffinose but without uracil were inoculated with the selected transformants and incubated at 30°C, 200 rpm for 2 days. 5 ml of CMdum liquid medium (without uracil) with 2% raffinose were then inoculated with the precultures to an OD_{600} of 0.05. Moreover, 0.2 mM γ-linolenic acid (GLA) was added in each case to the yeast culture transformed with pOTE1.1 and pOTE1.2. On the basis of the activity of OtELO1.1, an elongation of the γ-linolenic acid to the 20:3 fatty acid is to be expected. Respectively 0.2 mM arachidonic acid and eicosapentaenoic acid were added in each case to the yeast culture transformed with pOTE2.1 and pOTE2.2. Corresponding to the activity of OtELO2.1, it is to be expected that the fatty acids ARA and EPA will be elongated respectively to the 22:4 and 22:5 fatty acids. Expression was induced by adding 2% (w/v) galactose. The cultures were incubated at 20°C for a further 96 h.

Example 6: Expression of OtELO2.2 (as depicted in SEQ ID NO: 64) and OtELO1.2 (as in SEQ ID NO: 122) in yeasts

Yeasts transformed as in example 5 with the plasmids pYES2, pOTE1.2 and pOTE2.1 were analyzed in the following way:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0, in order to remove remaining medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acidic methanolysis. For this purpose, the cell sediments were incubated with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) dimethoxypropane at 80°C for 1 h. The FAMES were extracted by extraction twice with petroleum ether (PE). To remove underivatized fatty acids, the organic phases were washed once each with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and with 2 ml of distilled water. The PE phases were then dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C at a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with appropriate fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson (2001) Lipids 36(8): 761-766; Sayanova et al. (2001) Journal of Experimental Botany 52(360): 1581-1585, Sperling et al. (2001) Arch. Biochem. Biophys. 388(2): 293-298 and Michaelson et al. (1998) FEBS Letters 439(3): 215-218. The results of the analyses are depicted in table 1.

It was possible to confirm the appropriate activities both for pOTE1.1/pOTE1.2 and for pOTE2.1/2.2. The optimized sequence (respectively pOTE1.2 and pOTE2.2) showed activity in both cases. Synthesis of γ-linolenic acid could be increased only slightly by pOTE1.2 compared with the wild-type sequence. By contrast, it was possible to observe for pOTE2.2 surprisingly both an increase in the activity and an alteration in the specificity (table 1). In this connection, the activity for elongation of EPA had virtually doubled, while the elongation of ARA had more than quadrupled. It was thus possible with the optimization of the sequence of the Δ5-elongase from Ostreococcus tauri to increase the yield of the precursors of DHA 6-fold in yeast with the same amount of substrate.

Example 7: Cloning expression plasmids for the seed-specific expression in plants

The following general conditins described apply to all subsequent experiments unless described otherwise.

pBin19, pBI101, pBinAR, pGPTV, pCAMBIA or pSUN are preferably used for the following examples in accordance with the invention. An overview of the binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5: 446-451. A pGPTV derivative as described in DE10205607 was used. This vector differs from pGPTV by an additionally inserted AscI restriction cleavage site.

Starting point of the cloning procedure was the cloning vector pUC19 (Maniatis et al.). In the first step, the conlinin promoter fragment was amplified using the following primers:

Cnl1 C 5': gaattcggcgcgccgagctcctcgagcaacggttccggcggtatagagttgggtaattcga (SEQ ID NO: 200)

Cnl1 C 3': cccgggatcgatgccggcagatctccaccatttttttggtggtgat (SEQ ID NO: 201)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme EcoRI and then for 12 hours at 25°C with the restriction enzyme SmaI. The cloning vector pUC19 was incubated in the same manner. Thereafter, the PCR product and the 2668 bp cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C was verified by sequencing.

In the next step, the OCS terminator (Genbank Accession V00088; De Greve, H., et al. (1982) J. Mol. Appl. Genet. 1 (6): 499-511) was amplified from the vector pGPVT-USP/OCS (DE 102 05 607) using the following primers:

OCS_C 5': aggcctccatggcctgctttaatgagatatgcgagacgcc (SEQ ID NO: 202)

OCS_C 3': cccgggccggacaatcagtaaattgaacggag (SEQ ID NO: 203)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25°C with the restriction enzyme SmaI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_OCS was verified by sequencing.

In the next step, the Cnl1-B promoter was amplified by PCR by means of the following primers:

Cnl1-B 5': aggcctcaacggttccggcggtatag (SEQ ID NO: 204)

Cnl1-B 3': ccccggggttaacgctagcgggcccgatatcggatcccatttttttggtggtgattggttct (SEQ ID NO: 205)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25°C with the restriction enzyme SmaI. The vector pUC19-Cn11-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cn11B_OCS was verified by sequencing.

In a further step, the OCS terminator for Cnl1B was inserted. To this end, the PCR was carried out using the following primers:

OCS2 5': aggcctcctgctttaatgagatatgcgagac (SEQ ID NO: 206)

OCS2 3': cccgggcggacaatcagtaaattgaacggag (SEQ ID NO: 207)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 12 hours at 25°C with the restriction enzyme SmaI. The vector pUC19-Cnl1C_Cnl1B_OCS was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cnl1B_OCS2 was verified by sequencing.

In the next step, the Cnl1-A promoter is amplified by PCR using the following primers:

Cnl1-B 5': aggcctcaacggttccggcggtatagag (SEQ ID NO: 208)

Cnl1-B 3': aggccttctagactgcaggcggccgcccgcatttttggtggtgattggt (SEQ ID NO: 209)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme StuI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25°C with the restriction enzyme SmaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was verified by sequencing.

In a further step, the OCS terminator for Cnl1A was inserted. To this end, the PCR was carried out with the following primers:

OCS2 5': ggcctcctgctttaatgagatatgcga (SEQ ID NO: 210)

OCS2 3': aagcttggcgcgccgagctcgtcgacggacaatcagtaaattgaacggaga (SEQ ID NO: 211)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme StuI and then for 2 hours at 37°C with the restriction enzyme HindIII. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was incubated for 2 hours at 37°C with the restriction enzyme StuI and for 2 hours at 37°C with the restriction enzyme HindIII. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C_Cnl1B_Cnl1A_OCS3 was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the Δ6-, Δ5-desaturase and Δ6-elongase. To this end, the Phytium irregulare Δ6-desaturase (WO02/26946) was amplified using the following PCR primers:

D6Des(Pir) 5': agatctatggtggacctcaagcctggagtg (SEQ ID NO: 212)

D6Des(Pir) 3': ccatggcccgggttacatcgctgggaactcggtgat (SEQ ID NO: 213)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and then for 2 hours at 37°C with the restriction enzyme NcoI. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was incubated for 2 hours at 37°C with the restriction enzyme Bg*l*II and for 2 hours at 37°C with the restriction enzyme NcoI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir) was used for cloning the Thraustochytrium ssp. Δ5-desaturase (WO02/26946). To this end, the Thraustochytrium ssp. Δ5-desaturase was amplified using the following PCR primers:

D5Des(Tc) 5': gggatccatgggcaagggcagcgagggccg (SEQ ID NO: 214)

D5Des(Tc) 3': ggcgccgacaccaagaagcaggactgagatatc (SEQ ID NO: 215)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme BamHI and then for 2 hours at 37°C with the restriction enzyme *Eco*RV. The vector pUC19-Cnl1_d6Des(Pir) was incubated for 2 hours at 37°C with the restriction enzyme BamHI and for 2 hours at 37°C with the restriction enzyme EcoRV. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was used for cloning the Physcomitrella patens Δ6-elongase (WO01/59128), for which purpose the latter was amplified using the following PCR primers:

D6Elo(Pp) 5': gcggccgcatggaggtcgtggagagattctacggtg (SEQ ID NO: 216)

D6Elo(Pp) 3': gcaaaagggagctaaaactgagtgatctaga (SEQ ID NO: 217)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme NotI and then for 2 hours at 37°C with the restriction enzyme XbaI. The vector pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) was incubated for 2 hours at 37°C with the restriction enzyme NotI and for 2 hours at 37°C with the restriction enzyme XbaI. Thereafter, the PCR product and cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was verified by sequencing.

The binary vector for the transformation of plants was prepared starting from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp). To this end, pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was incubated for 2 hours at 37°C with the restriction enzyme AscI. The vector pGPTV was treated in the same manner. Thereafter, the fragment from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) and the cleaved pGPTV vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) was verified by sequencing.

A further construct, pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co), was used. To this end, the amplification was carried out with the following primers, starting from pUC19-Cnl1C_OCS:

Cnl1_OCS 5': gtcgatcaacggttccggcggtatagagttg (SEQ ID NO: 218)

Cnl1_OCS 3': gtcgatcggacaatcagtaaattgaacggaga (SEQ ID NO: 219)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme SalI. The vector pUC19 was incubated for 2 hours at 37°C with the restriction enzyme SalI. Thereafter, the PCR product and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_OCS was verified by sequencing.

In a further step, the Calendula officinalis Δ12-desaturase gene (WO01/85968) was cloned into pUC19-Cnl1_OCS. To this end, d12Des(Co) was amplified with the following primers:

D12Des(Co) 5': agatctatgggtgcaggcggtcgaatgc (SEQ ID NO: 220)

D12Des(Co) 3': ccatggttaaatcttattacgatacc (SEQ ID NO: 221)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM $MgCl_2$

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme BglII and thereafter for 2 hours at the same temperature with NcoI. The vector pUC19-Cnl1_OCS was incubated in the same manner. Thereafter, the PCR fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_D12Des(Co) was verified by sequencing.

The plasmid pUC19-Cnl1_D12Des(Co) and the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) were incubated for 2 hours at 37°C with the restriction enzyme SalI. Thereafter, the vector fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was

purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and vector fragment were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was verified by sequencing.

The binary vector for the transformation of plants was prepared starting from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co). To this end, pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was incubated for 2 hours at 37°C with the restriction enzyme *Asc*I. The vector pGPTV was treated in the same manner. Thereafter, the fragment from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) and the cleaved pGPTV vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pGPTV- Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was verified by sequencing.

A further example of the use of seed-specific expression constructs is the Napin promoter. Preparation of these expression constructs in the vectors pGPTV or pSUN is described in Wu et al. (2005) Nat. Biotech. 23:1013-1017.

A further vector suitable for plant transformation is pSUN2. This vector was used in combination with the Gateway system (Invitrogen, Karlsruhe) in order to increase the number of expression cassettes present in the vector to more than four. For this purpose, the Gateway cassette A was inserted into the vector pSUN2 in accordance with the manufacturer's instructions, as described below:

The pSUN2 vector (1 µg) was incubated with the restriction enzyme EcoRV at 37° for 1 h. The Gateway cassette A (Invitrogen, Karlsruhe) was then ligated into the cut vector using the Rapid Ligation kit from Roche, Mannheim. The resulting plasmid was transformed into E. coli DB3.1 cells (Invitrogen). The isolated plasmid pSUN-GW was then verified by sequencing.

In the second step, the expression cassette was cut out of pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) using AscI and ligated into the likewise treated vector pSUN-GW. The plasmid obtained in this way pSUN-4G was used for further gene

constructs.

For this purpose, firstly a pENTR clone was modified in accordance with the manufacturer's instructions (Invitrogen). The plasmid pENTR1A (Invitrogen) was incubated with the restriction enzyme EcoRI at 37° for 1 h and then treated with Klenow enzyme and with a 1 µM dNTP mix for 30 min, and subsequently the AscI adapter (5'-ggcgcgcc; phosphorylated at the 5' end, double-stranded) was ligated into the pENTR1A vector. Genes were inserted as described above stepwise into the Cnl cassette in these modified and transferred via AscI into the pENTR vector, resulting in the pENTR-Cnl vector.

In a further step, the pSUN-8G construct was prepared. For this purpose, 5' and 3' primers for the genes with the SEQ ID NOs: 1, 3, 5 and 7 with the restriction cleavage sites described above and with the first and in each case last 20 nucleotides of the open reading frame were produced and amplified with the standard conditions (see above) and ligated into the pENTR-Cnl vector, which was subsequently subjected to a recombination reaction with the pSUN-4G vector in accordance with the manufacturer's instructions.

The construct pSUN-8G was prepared in this way and was transformed into *Brassica juncea* and Brassica napus. The seeds of the transgenic plants were analyzed by gas chromatography.

A further construct which was used for transformation of *B. juncea* and *B. napus* was the construct pSUN-9G. This construct was prepared according to Wu et al. (2005) Nat. Biotech. 23:1013-1017 with the napin promoter. In a modification of Wu et al. 2005, the coding sequence of OtELO2.2 was inserted in the described manner instead of the gene OmELO. The resulting construct pSUN-9G was then transformed into *B. juncea* and *B. napus*.

Example 8: Lipid extraction from plant material

The effect of the genetic modification in plants on the production of a desired compound (such as a fatty acid) can be determined by growing the modified plant under suitable conditions (such as those described above) and analyzing the medium and/or the cellular components for the elevated production of the desired product (i.e. of the lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various types of staining methods, enzymatic and microbiological methods and analytical chromatography such as high-performance liquid chromatography (see, for example,

Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon A. et al. (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", p. 469-714, VCH: Weinheim; Belter, P.A. et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., and Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., and Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. B3; Chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned methods, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940 and Browse et al. (1986) Analytic Biochemistry 152:141-145. The qualitative and quantitative analysis of lipids or fatty acids is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

In addition to measuring the end product of the fermentation, it is also possible to analyze other components of the metabolic pathways which are used for the production of the desired compound, such as intermediates and by-products, in order to determine the overall production efficiency of the compound. The analytical methods comprise measuring the amount of nutrients in the medium (for example sugars, hydrocarbons, nitrogen sources, phosphate and other ions), measuring the biomass composition and the growth, analyzing the production of conventional metabolytes of biosynthetic pathways and measuring gases which are generated during the fermentation. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, Ed., IRL Press, p. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAME, fatty acid methyl ester; GC-MS, gas liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

The unambiguous detection for the presence of fatty acid products can be obtained by analyzing recombinant organisms using analytical standard methods: GC, GC-MS or TLC, as described on several occasions by Christie and the references therein (1997, in: Advances on Lipid Methodology, Fourth Edition: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren [Gas chromatography/mass spectrometric methods], Lipide 33:343-353).

The material to be analyzed can be disrupted by sonication, grinding in a glass mill, liquid nitrogen and grinding or via other applicable methods. After disruption, the material must be centrifuged. The sediment is resuspended in distilled water, heated for 10 minutes at 100°C, cooled on ice and recentrifuged, followed by extraction for one hour at 90°C in 0.5 M sulfuric acid in methanol with 2% dimethoxypropane, which leads to hydrolyzed oil and lipid compounds, which give transmethylated lipids. These fatty acid methyl esters are extracted in petroleum ether and finally subjected to a GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) at a temperature gradient of between 170°C and 240°C for 20 minutes and 5 minutes at 240°C. The identity of the resulting fatty acid methyl esters must be defined using standards which are available from commercial sources (i.e. Sigma).

Plant material is initially homogenized mechanically by comminuting in a pestle and mortar to make it more amenable to extraction.

This is followed by heating at 100°C for 10 minutes and, after cooling on ice, by resedimentation. The cell sediment is hydrolyzed for one hour at 90°C with 1 M methanolic sulfuric acid and 2% dimethoxypropane, and the lipids are transmethylated. The resulting fatty acid methyl esters (FAMEs) are extracted in petroleum ether. The extracted FAMEs are analyzed by gas liquid chromatography using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) and a temperature gradient of from 170°C to 240°C in 20 minutes and 5 minutes at 240°C. The identity of the fatty acid methyl esters is confirmed by comparison with corresponding FAME standards (Sigma). The identity and position of the double bond can

be analyzed further by suitable chemical derivatization of the FAME mixtures, for example to give 4,4-dimethoxyazolin derivatives (Christie, 1998) by means of GC-MS.

Example 9: Use of the optimized Δ5-elongase (as depicted in SEQ ID NO: 64) from *Ostreococcus tauri* for constructs for constitutive expression

Transformation vectors based on pGPTV-35S, a plasmid based on pBIN19-35S (Bevan M. (1984) Nucl. Acids Res. 18:203), were produced for the transformation of plants. For this purpose, firstly an expression cassette consisting of the promoter element CaMV35S (SEQ ID NO: 161) and the 35S terminator (SEQ ID NO: 162; Franck, A. et al. (1980) Cell 21 (1): 285-294) was assembled in a pUC vector. This entailed the promoter being inserted via the SalI/XbaI restriction cleavage sites and the terminator via the BamHI/SmaI restriction cleavage sites. In addition, a polylinker with the XhoI cleavage site was attached to the terminator ('triple ligation'). The resulting plasmid pUC19-35S was then employed for cloning PUFA genes. In parallel, the open reading frames of the Δ6-desaturase (SEQ ID NO: 1), of the Δ5-desaturase (SEQ ID NO: 51) and Δ6-elongase (SEQ ID NO: 171) sequences were inserted via the EcoRV cleavage site into pUC19-35S vectors. The resulting plasmids pUC-D6, pUC-D5, pUC-E6(Tc) were used to construct the binary vector pGPTV-35S_D6D5E6(Tc). For this purpose, the vector pGPTV was digested with the enzyme SalI, the plasmid pUC-D6 was digested with SalI/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6 was then digested with SalI, the plasmid pUC-D5 was digested with SalI/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6-D5 was then digested once more with SalI, the plasmid pUC-E6(Tc) with SalI/XhoI, and the correct fragments were ligated. These sequential cloning steps resulted in the binary vector pGPTV-D6D5E6(Tc), which was employed for the transformation.

In a further procedure, the sequence of d6Elo(Tp) (SEQ ID NO: 163) was inserted into the vector pUC19-35S instead of the sequence d6Elo(Tc). The resulting plasmid pUC-E6(Tp) was used to prepare the binary vector pGPTV-35S_D6D5E6(Tp).

In a further procedure, the open reading frame of ω3Des (SEQ ID NO: 193) was cloned into pUC19-35S. The resulting plasmid pUC-ω3Pi was transferred via SalI/XhoI into the binary vectors pGPTV-D6D5E6(Tc) and pGPTV-D6D5E6(Tp). The resulting vectors pGPTV-D6D5E6(Tc)ω3Pi and pGPTV-D6D5E6(Tp)ω3Pi were employed for the plant transformation.

In a further procedure, the open reading frame of the optimized Δ5-elongase from *Ostreococcus tauri* (SEQ ID NO: 64) and the open reading frame of the Δ4-desaturase from *Thraustochytrium* sp. (SEQ ID NO: 77) was cloned into pUC19-35S. The resulting plasmids pUC-E5 and pUC-D4 were then transferred via SalI/XhoI in accordance with the above statements into the vector pGPTV-D6D5E6(Tp)ω3Pi. The resulting vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was employed for the plant transformation.

All the binary vectors were transformed into *E. coli* DH5α cells (Invitrogen) in accordance with the manufacturer's instructions. Positive clones were identified by PCR, and plasmid DNA was isolated (Qiagen Dneasy).

Example 10: Transformation of the constitutive binary vectors into plants

a) Generation of transgenic *Brassica napus* and *Brassica juncea* plants. The protocol for the transformation of oilseed rape plant was used (modification of Moloney et al. (1992) Plant Cell Reports 8:238-242)

The binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed in *Agrobacterium tumefaciens* C58C1:pGV2260 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Murashige and Skoog (1962) Physiol. Plant. 15: 473) supplemented with 3% sucrose (3MS medium) was used for the transformation of *Orychophragmus violaceus*. Petioles or hypocotyls of freshly germinated sterile plants (in each case approx. 1 cm^2) were incubated with a 1:50 agrobacterial dilution for 5-10 minutes in a Petri dish. This is followed by 3 days of coincubation in the dark at 25°C on 3MS medium supplemented with 0.8% Bacto agar. Thereafter, the cultivation was continued with 16 hours light/8 hours dark and a weekly rhythm on MS medium supplemented with 500 mg/l Claforan (cefotaxime-sodium), 50 mg/l kanamycin, 20 µM benzylaminopurine (BAP) and 1.6 g/l glucose. Growing shoots were transferred to MS medium supplemented with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan, then, after rooting, transferred into soil and, after cultivation, grown for two weeks in a controlled-environment cabinet or in the greenhouse, allowed to flower, mature seeds were harvested and

analyzed for elongase expression such as Δ6-elongase activity or for Δ5- or Δ6-desaturase activity by means of lipid analyses. In this manner, lines with elevated contents of polyunsaturated C20- and C22-fatty acids were identified.

b) Generation of transgenic *Orychophragmus violaceus* plants

The protocol for the transformation of oilseed rape plants was used (modification of Moloney et al. (1992) Plant Cell Reports 8:238-242) as described under a).

To generate transgenic plants, the binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was transformed into *Agrobacterium tumefaciens* C58C1:pGV2260 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed *Agrobacterium* colony in Murashige-Skoog medium (Murashige and Skoog (1962) Physiol. Plant, 15: 473) with 3% sucrose (3MS medium) was used to transform *Orychophragmus violaceus*. Petioles or hypocotyls of freshly germinated sterile plants (each about 1 cm^2) were incubated with a 1:50 agrobacterial dilution in a Petri dish for 5-10 minutes. This is followed by coincubation on 3MS medium with 0.8% Bacto agar in the dark at 25°C for 3 days. The cultivation was then continued with 16 hours light/8 hours dark and in a weekly rhythm on MS medium with 500 mg/l Claforan (cefotaxime sodium), 15 mg/l kanamycin, 20 µM benzylaminopurine (BAP) and 1.6 g/l glucose. Growing shoots were transferred to MS medium with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots had developed after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan and, after rooting, transferred to soil and, after cultivation, grown for two weeks in a controlled environment cabinet or in a greenhouse, allowed to flower, and mature seeds were harvested and examined by lipid analyses for elongase expression such as Δ6-elongase activity or Δ5- or Δ6-desaturase activity. Lines with increased contents of polyunsaturated C20 and C22 fatty acids were identified in this way.

c) Transformation of *Arabidopsis thaliana* plants

The protocol of Bechthold et al. (1993) C.R. Acad. Sci. Ser. III Sci. Vie. 316: 1194-1199 was used.

To generate transgenic plants, the generated binary vector pGPTV-D6D5E6(Tp)ω3PiE5D4 was

transformed into *Agrobacterium tumefaciens* C58C1:pMP90 (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788) and, in accordance with the protocol of Bechthold et al. (1993), flowers of *Arabidopsis thaliana* cv. Columbia 0 were dipped in an agrobacterial solution with OD600=1.0. The procedure was repeated again two days later. Seeds from these flowers were then placed on agar plates with ½ MS, 2% sucrose and 50 mg/l kanamycin. Green seedlings were then transferred to soil.

Example 11: Analysis of plant material of transgenic *Orychophragmus* or *Arabidopsis* plants

Extraction of leaf material of transgenic *Orychophragmus violaceus* and *Arabidopsis thaliana* plants transformed with pGPTV-D6D5E6(Tp)ω3PiE5D4 and the gas chromatography analysis was carried out as described in example 8. Table 2 shows the results of the analyses. The various fatty acids are indicated in percent by weight. It was possible to show that long-chain polyunsaturated fatty acids were synthesized by both different plant species. It was surprisingly possible with the optimized sequence of the Δ5-elongase (as depicted in SEQ ID NO: 64) from *Ostreococcus tauri* to obtain a distinctly higher yield of DHA than reported for example by Robert et al. (2005) Functional Plant Biology 32: 473-479 for Arabidopsis thaliana with 1.5% DHA. It was possible for the first time to achieve a synthesis of long-chain polyunsaturated fatty acids for *Orychophragmus violaceus*.

Example 12: Analysis of seeds of transgenic *Brassica juncea* lines

Extraction of seeds of transgenic *Brassica juncea* plants transformed with pSUN-9G, and the gas chromatography analysis was carried out as described in example 8. Table 6 shows the results of the analyses. The various fatty acids are indicated in percent area. As in Wu et al. 2005 it was possible to show the synthesis of long-chain polyunsaturated fatty acids (PUFA). Surprisingly, the use of the modified elongase sequence OtELO2.2 such as the nucleic acid sequence described by SEQ ID NO: 64 resulted in a drastic increase in the content of C22 fatty acids. In total, the seed oil contained about 8% by weight % polyunsaturated C22 fatty acids. Specifically, the content of the fatty acid docosahexaenoic acid (DHA) in the seed oil was 1.9% by weight %, representing an increase by a factor of 10 compared with Wu et al. 2005.

Example 13: Detailed analysis of the lipid classes and position analysis of leaf material from *O. violaceus*

About 1 g of leaf tissue was heated in 4 ml of isopropanol at 95°C for 10 minutes, homogenized by Polytron and shaken after addition of 1.5 ml of chloroform. The samples were centrifuged, the supernatant was collected, and the pellet was extracted again with isopropanol:chloroform 1:1 (v/v). The two extracts were combined, dried and dissolved in chloroform. The lipid extract was prefractionated on a silica prepsep column (Fisher Scientific, Nepean, Canada) into neutral lipids, glycolipids and phospholipids, eluting with chloroform:acetic acid 100:1 (v/v), acetone:acetic acid 100:1 (v/v) and methanol:chloroform:water 100:50:40 (v/v/v), respectively. These fractions were further fractionated on silica G-25 thin-layer chromatography plates (TLC; Macherey-Nagel, Düren, Germany). Neutral lipids were developed with hexane:diethyl ether:acetic acid (70:30:1), glycolipids with chloroform:methanol:ammonia (65:25:4 v/v/v) and phospholipids with chloroform:methanol:ammonia:water (70:30:4:1 v/v/v/v). The individual lipid classes were identified after spraying with primulin under UV light, removed by scraping off the plates and either used for direct transmethylation or extracted by a suitable solvent for further analysis.

It was possible by the disclosed methods for the various lipid classes (neutral lipids, phospholipids and galactolipids) to be fractionated and analyzed separately. The glycolipids were additionally examined for the position of the individual fatty acids.

a) Regiospecific analysis of the triacylglycerides (TAG)

Three to five mg of the TLC-purified TAG were dried under nitrogen in a glass tube, resuspended in aqueous buffer by brief ultrasound treatment (1 M Tris pH 8; 2.2% $CaCl_2$ (w/v); 0.05% bile salts (w/v)) and incubated at 40°C for 4 minutes. After addition of 0.1 ml of a solution of pancreatic lipase (10 mg/ml in water), the samples were vigorously vortexed for 3 minutes, and the digestion was stopped by adding 1 ml of ethanol and 1.5 ml of 4 M HCl. The partly digested TAGs were extracted twice with diethyl ether, washed with water, dried and dissolved in a small volume of chloroform. Monoacylglycerols (MAG) were separated from the free fatty acids and undigested TAGs on a TLC plate as described above for neutral lipids. The point corresponding to the MAGs was analyzed by GC and represented the sn-2 position of the TAGs. The distribution of the fatty acids to the remaining sn-1 and sn-3 positions was calculated by the following formula: sn-1 + sn-3 = (TAG × 3 - MAG)/2.

This position analysis of the triacylglycerides revealed in this case that EPA and DHA are

present in similar concentrations in the sn-2 and sn-1/3 positions, while ARA is to be found overall only in small amounts in the triacylglycerides, and here mainly in the sn-2 position (Tab. 3).

b) Stereospecific analysis of phospholipids

Fractionated and extracted phosphatidylglycol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were dried under N_2 and resuspended in 0.5 ml of borate buffer (0.5M, pH 7.5, containing 0.4 mM $CaCl_2$). After a brief ultrasound treatment, 5U of phospholipase A2 from the venom of *Naja mossambica* (Sigma P-7778) and 2 ml of diethyl ether were added and the samples were vortexed at room temperature for 2 hours. The ether phase was dried, the digestion was stopped with 0.3 ml of 1M HCl, and the reaction mixture was extracted with chloroform:methanol (2:1 v/v). The digested phospholipids were separated by TLC in chloroform:methanol:ammonia:water (70:30:4:2 v/v/v/v) and points which corresponded to the liberated free fatty acids and lysophospholipids were removed by scraping and directly transmethylated.

Positional analysis of the phospholipids showed an accumulation of EPA and DHA in the sn-2 position of phosphatidylcholine (PC), while DHA was similarly distributed in sn-1 and sn-2 position in phosphatidylethanolamine (PE). Only traces of, or no, ARA was to be found in both phospholipids (Tab. 4). The concentrations of EPA and DHA in phosphatidylglycerol were lower than in the other investigated phospholipids, with accumulation in the sn-2 position also to be observed in this lipid class (Tab. 4, PG).

c) Stereospecific analysis of glycolipids

The galactolipids were investigated as a further polar lipid class. Galactolipids are found in the membranes of plastids and form the main components there.

TLC-purified monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were dried under nitrogen and dissolved in 0.5 ml of diethyl ether. Then 25 units of the lipase from Rhizopus arrhizus (Sigma 62305), resuspended in 2 ml of borate buffer (50 mM, pH 7.5 containing 2 mM $CaCl_2$), were added, and the samples were vortexed at room temperature for 2 hours. The ether phase was dried and the digestion was stopped by adding 0.3 ml of 1M HCl, and the lipids were extracted with 4 ml of chloroform:methanol (2:1 v/v). After drying, the

digested galactolipids were in a small volume of chloroform:methanol (2:1 v/v) and developed twice on a precoated silica TLC plate, firstly with chloroform:methanol: ammonia:water (70:30:4:1 v/v/v/v) to about two thirds the height of the plate, followed by complete development in hexane:diethyl ether:acetic acid (70:30:1). The points which corresponded to the liberated free fatty acids and the lysogalactolipids were identified after spraying with primulin, scraped off and transmethylated directly for GC analysis.

It was possible to find VLCPUFA (very long chain polyunsaturated fatty acid) in these lipids too, with an accumulation of EPA in the sn-2 position being observed. DHA was to be found only in the digalactodiacylglycerols (DGDG) and was undetectable in the monogalactodiacylglycerols (MGDG) (Table 5). The distribution of VLCPUFA in galactolipids, a compartment in which these fatty acids were not expected, shows the dynamics of the synthesis and the later transformation. VLCPUFA in polar lipids are of particular nutritional value because they can be absorbed better in the intestines of mammals than the neutral lipids.

Table 1: Test of the optimized sequences of pOTE1.1 and pOTE2.1 in yeast. The conversion rates were determined in accordance with the substrate conversions. A distinct rise in activity was achievable with the optimized sequence in plasmid pOTE2.2.

Conversion rates of the *Ostreococcus tauri* elongases					
	Genes	**Substrate** **Product**	**GLA** **20:3**	**ARA** **22:4**	**EPA** **22:5**
pOTE1.1	**d6Elongase(Ot)**		**21.1**		
pOTE1.2	**d6Elongase(Ot)_opt**		**25.6**		
pOTE2.1	**d5Elongase(Ot)**			**7.3**	**35.9**
pOTE2.2	**d5Elongase(Ot)_opt**			**32.7**	**63.1**

Table 2: Gas chromatographic analysis of leaf material of *Orychophragmus violaceus* and *Arabidopsis thaliana*. The individual fatty acids are indicated in percent area.

Fatty acid composition of leaf material of *Orychophragmus violaceus*											
Fatty acids	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Control	20.9	8.5	3.3	16.0	0.0	47.4	0.0	0.0	0.0	0.0	0.0
Transgene	21.3	8.2	5.2	5.2	4.2	23.1	5.0	0.6	13.5	2.7	4.5

Fatty acid composition of leaf material of *Arabidopsis thaliana*											
Fatty acids	16:0	16:3	18:1	18:2	GLA	18:3	18:4	ARA	EPA	DPA	DHA
Control	12.8	10.0	3.5	14.2	0.0	54.6	0.0	0.0	0.0	0.0	0.0
Transgene	19.3	8.5	5.0	4.6	6.4	31.0	4.4	0.0	6.3	1.5	6.3

Table 3: Regiospecific analysis of the triacylglycerides from leaf material from transgenic *O. violaceus* plants.

TAG	16:0	18:0	18:1n-9	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
wt	25.12	3.03	5.06		18.53		44.72							
sn-2	1.42	0.76	6.79		27.62		62.03							
sn-1+3	36.97	4.17	4.19		13.98		36.07							
Transgene	22.63	3.12	3.45	0.77	2.35	9.51	6.37	13.03	0.74	0.83	3.87	24.96	2.22	4.15
sn-2	1.62	0.64	8.33	1.61	5.15	16.21	10.88	19.84	0.17	1.38	1.99	24.82	3.27	3.02
sn-1+3	33.13	4.36	1.02	0.35	0.96	6.16	4.11	9.63	1.02	0.55	4.80	25.03	1.69	4.72

Table 4: Stereospecific analysis of the phospholipids from leaf material from transgenic O. violaceus plants.

PG	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
wt	27,96	20,04	4,11	2,89	0,90		21,82	0,00	21,56							
sn-2	17,26	0,53	2,61	3,82	1,91		39,01	0,00	34,44							
sn-1	38,66	39,56	5,62	1,96	0,00		4,62	0,00	8,69							
Transgene	27,15	24,70	3,08	4,62	1,20	0,00	15,15	1,53	17,94	1,40	0,00	0,00	0,45	2,18	0,10	0,58
sn-2	21,16	3,61	4,23	7,52	2,14		27,40	0,50	31,57	0,81			0,38	1,24	0,00	0,33
sn-1	33,15	45,79	1,94	1,71	0,27		2,90	2,57	4,30	2,00			0,51	3,13	0,27	0,83

PE	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
wt	37,49	0,00	6,62	4,35	1,37		19,28		29,95							
sn-2	54,22	0,00	7,74	3,39	3,42		12,64		13,71							
sn-1	20,77	0,00	5,51	5,31	0,00		25,93		46,18							
Transgene	31,78	0,81	5,84	3,08	2,20	0,85	5,57	11,25	11,34	7,38	0,00	0,00	2,88	9,41	1,90	4,90
sn-2	50,17	0,33	10,86	3,22	4,94	0,35	2,63	3,27	3,59	2,31	0,56		4,42	6,18	0,38	4,19
sn-1	13,40	1,29	0,83	2,95	0,00	1,35	8,50	19,23	19,10	12,45	0,00		1,34	12,64	3,41	5,61

PC	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:5n-3	22:6n-3
wt	27,67	0,84	6,38	8,56	1,80		21,75		33,01							
sn-2	48,05	0,44	8,65	5,05	3,41		14,52		18,04							
sn-1	7,28	1,24	4,11	12,06	0,18		28,97		47,98							
Transgene	21,00	0,00	8,01	10,02	2,86	1,25	3,77	11,63	5,60	12,11	0,50	0,00	4,34	11,16	3,76	3,70
sn-2	45,35	0,00	14,71	5,08	5,70	0,31	3,23	3,09	4,58	2,65	0,61	0,08	4,01	8,32	0,41	1,18
sn-1	3,36	0,00	1,30	14,96	0,02	2,20	4,31	20,18	6,62	21,56	0,38	0,00	4,66	13,99	7,12	6,22

Table 5: Stereospecific analysis of the galactolipids from leaf material from transgenic O. violaceus plants.

MGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**2,64**	**0,13**	**1,23**	**30,72**	**0,33**	**0,35**	**0,26**		**3,81**		**60,52**					
sn-2	0,00	0,05	0,00	7,11	0,35	0,31	0,41		4,60		87,30					
sn-1	5,34	0,21	2,55	54,34	0,31	0,39	,012		3,01		33,74					
Transgene	**4,16**	**0,20**	**1,08**	**33,81**	**0,93**	**0,73**	**0,52**	**0,03**	**1,64**	**1,88**	**44,82**	**2,73**	**0,04**	**0,30**	**0,50**	**5,08**
sn-2	1,22	0,29	0,54	4,79	1,51	1,15	0,93	0,00	2,80	0,14	80,19	0,00	0,08	0,17	0,87	3,86
sn-1	7,11	0,11	1,61	62,82	0,34	0,31	0,11	0,11	0,47	3,62	9,46	5,48	0,00	0,43	0,14	6,31

DGDG	16:0	16:1	16:2	16:3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:3n-6	20:4n-6	20:4n-3	20:5n-3
wt	**17,67**	**0,19**	**0,38**	**2,15**	**1,61**	**0,51**	**0,94**		**5,56**		**70,71**					
sn-2	16,84	0,25	0,50	2,52	2,21	0,55	1,75		6,07	0,00	68,74					
sn-1	18,50	0,12	0,27	1,78	1,01	0,46	0,13		5,05		72,68					
Transgene	**18,50**	**0,00**	**0,00**	**2,62**	**2,84**	**1,36**	**1,39**	**0,00**	**6,28**	**3,55**	**54,66**	**0,00**	**0,00**	**0,00**	**2,18**	**5,36**
sn-2	22,74	0,17	0,23	0,48	4,55	1,71	2,32	0,24	9,22	0,23	56,06	0,27	0,00	0,00	0,36	1,23
sn-1	14,27	0,00	0,00	4,77	1,12	1,00	0,46	0,00	3,33	6,88	53,26	0,00	0,00	0,00	4,01	9,49

Table 6: Gas chromatographic determination of the fatty acids from seeds of transgenic Brassica juncea plants transformed with the construct pSUN-9G in percent by weight. WT describes the unmodified wild-type control.

	Lipid Profile (%)							
	16:0	18:0	18:1	18:2	γ18:3	α18:3	18:4	20:0
BJ223_PUFA184_MKP71_581A	4,4	3,0	22,5	16,9	27,0	4,9	3,2	0,6
BJ223_PUFA184_MKP71_581A	4,7	3,9	17,9	10,6	29,5	4,2	4,0	0,9
BJ223_PUFA184_MKP71_581A	4,4	3,0	18,9	13,8	30,5	4,1	3,2	0,7
BJ223_PUFA184_MKP71_581A	4,6	3,3	20,5	13,2	29,8	4,2	3,3	0,8

20:3 (8,11,14)	20:3 (11, 14, 17)	20:4 (ARA) (5,8,11,14)	20:4 (ETeA) (8,11,14,17)	20:5 (EPA) (5,8,11,14,17)	22:1	22:4	22:5	22:6
1,1	0,5	3,1	0,6	4,6	0,0	1,5	2,0	1,5
2,0	0,9	4,2	1,0	4,1	0,0	3,1	3,5	1,9
1,3	0,7	4,1	0,5	4,5	0,0	2,7	2,8	1,6
1,4	0,6	3,6	0,6	4,4	0,0	2,4	2,5	1,6

Electronic Acknowledgement Receipt

EFS ID:	34868042
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Bronwen M. Loeb/Felicia Bull
Filer Authorized By:	Bronwen M. Loeb
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Time Stamp:	11:09:14
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		074017_0013_01_RN_NTFCAP.pdf	2138530 ea8b55cb5217c45058762cc9dfed8830669956aa	yes	5

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	Document Description	**Start**	**End**
	Applicant Response to Pre-Exam Formalities Notice	1	1
	Specification	2	2
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2	Specification	SubSpecClean_1_88.pdf	2065640 6ca984fa7753e4318aea72be5cf8f67a3f1b1723	no	88
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New International Application Filed with the USPTO as a Receiving Office
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(Signature)
(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
15/256,914	09/06/2016	Petra CIRPUS	074017-0013-01-US-541474	4050

TITLE OF INVENTION: METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1,000.00	$0	$0	$1,000.00	March 19, 2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
H. A. Robinson	1652	514-558000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Drinker Biddle & Reath LLP
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3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

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(A) NAME OF ASSIGNEE: BASF Plant Science GmbH

(B) RESIDENCE: (CITY and STATE or COUNTRY): Ludwigshafen, Germany

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

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5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29. NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27. NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

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Authorized Signature: /Bronwen M Loeb/ Date: March 15, 2019

Typed or printed name: Bronwen M. Loeb, Ph.D. Registration No. 43,516

Electronic Patent Application Fee Transmittal

Application Number:	15256914
Filing Date:	06-Sep-2016
Title of Invention:	OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT
First Named Inventor/Applicant Name:	Petra Cirpus
Filer:	Bronwen M. Loeb/Jamie Jensen-Smith
Attorney Docket Number:	074017-0013-01-US

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
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Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	1000	1000

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1000**

Electronic Acknowledgement Receipt

EFS ID:	35434298
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
Title of Invention:	OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT
First Named Inventor/Applicant Name:	Petra Cirpus
Customer Number:	123223
Filer:	Bronwen M. Loeb/Jamie Jensen-Smith
Filer Authorized By:	Bronwen M. Loeb
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	15-MAR-2019
Filing Date:	06-SEP-2016
Time Stamp:	11:45:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1000
RAM confirmation Number	031519INTEFSW11455500
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	IssueFeeTransmittal.pdf	17475 95562e22525b92e8332d8bb9e3758f1a16c340ac	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30565 5c179f7f48285334da8317ea87cd3dd8eeea47cb	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	48040		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	09/06/2016	Petra Cirpus	074017-0013-01-US	4050

123223 7590 04/26/2019

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

EXAMINER	
ROBINSON, HOPE A	

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
04/26/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DBRIPDocket@dbr.com
IPDocketWM@dbr.com

Response to Rule 312 Communication	Application No. 15/256,914	Applicant(s)
	Examiner	Art Unit

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address –

1. ☒ The amendment filed on *16 January 2019* under 37 CFR 1.312 has been considered, and has been:

a) ☒ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

/ N. Horne /

PUBLISHING DIVISION

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /H.A.R/

PTO/SB/08b (07-09)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	15/256,914 -- Conf.#4050
Filing Date	September 6, 2016
First Named Inventor	Petra CIRPUS
Art Unit	1652
Examiner Name	Hope A. Robinson
Attorney Docket Number	074017-0013-01-US

Change(s) applied to document, /N.B.H./ 1/5/2019

U. S. PATENT DOCUMENTS

Examiner Initials	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		20150361404	12-17-2015	~~BASF Plant Science GmbH~~ Cirpus et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	WAGNER, et al., "Generation of glycerophospholipid molecular species in the yeast Saccharomyces cerevisiae. Fatty acid pattern of phospholipid classes and selective acyl turnover at sn-1 and sn-2 positions", Yeast, Vol. 10, 1994, pp. 1429-1437	
	CB	DIEDRICH, et al., "The natural occurrence of unusual fatty acids. Part 1. Odd numbered fatty acids", Molecular Nutrition & Food Research, Vol. 34, Issue 10, 1990, pp. 935-943	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	10/18/2018

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

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Alexandria, Virginia 22313-1450
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	05/28/2019	10301638	074017-0013-01-US	4050

123223 7590 05/08/2019

Drinker Biddle & Reath LLP (WM)
222 Delaware Avenue, Ste. 1410
Wilmington, DE 19801-1621

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

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