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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:

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:

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/98 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 Popp	Telephone	
Title	Dr. Uwe Pfeiffer		

Vice Presidents

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

Drinker Biddle & Reath LLP

222 Delaware Ave., Ste. 1410

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(302) 351-6938 (Fax)

Attorney for Applicant

#85,080,132

UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	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 <i>See MPEP chapter 600 concerning utility patent application contents.</i>	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450
--	--

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. **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. A copy from a prior application (37 CFR 1.63(d))
7. **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

ACCOMPANYING APPLICATION PAPERS	
10. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) Name of Assignee <div style="border: 1px solid black; height: 20px; width: 100%;"></div>	11. <input type="checkbox"/> 37 CFR 3.73(c) Statement <i>(when there is an assignee)</i>
	<input checked="" type="checkbox"/> Power of Attorney
	12. <input type="checkbox"/> English Translation Document <i>(if applicable)</i>
	13. <input checked="" type="checkbox"/> Information Disclosure Statement (PTO/SB/08 or PTO-1449) <input type="checkbox"/> Copies of citations attached
	14. <input type="checkbox"/> Preliminary Amendment
	15. <input type="checkbox"/> Return Receipt Postcard <i>(MPEP § 503) (Should be specifically itemized)</i>
	16. <input type="checkbox"/> Certified Copy of Priority Document(s) <i>(if foreign priority is claimed)</i>
	17. <input type="checkbox"/> Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
	18. <input checked="" type="checkbox"/> Other: Text File of Sequence Listing Submitted via EFS Web <div style="border: 1px solid black; height: 20px; width: 100%;"></div>

***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

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

Application Data Sheet

Inventor Information

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

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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 _____.

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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
06120309.7	Europe	07 September 2006	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

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
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Date 4.5.2007

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J. Bauer
Signature of Inventor

Date 04.05.2007

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Date y March 29, 2007

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Date y March 29, 2007

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Date y March 29, 2007

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x Martin Truksa

Signature of Inventor

Date *x April 12, 2007*

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Citizen of Germany
Post Office Address: same as residence

Tom Wejen
09.05.2007
Teckla Bopius

Signature of Inventor

Date *4.5.2007*

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 1449A/B/PTO		Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)		Application Number	Not Yet Assigned
		Filing Date	Concurrent Herewith
		First Named Inventor	Petra Cirpus
		Art Unit	N/A
		Examiner Name	Not Yet Assigned
Sheet	1	of	6
		Attorney Docket Number	074017-0013-01-US

U.S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)				
	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		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
		Country Code ³ -Number ² - Kind Code ² (if known)					
	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 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.

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 1449A/B/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Application Number	Not Yet Assigned
				Filing Date	Concurrent Herewith
				First Named Inventor	Petra Cirpus
				Art Unit	N/A
				Examiner Name	Not Yet Assigned
Sheet	2	of	6	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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				Art Unit	N/A
				Examiner Name	Not Yet Assigned
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NON PATENT LITERATURE DOCUMENTS			
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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.	
	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, <i>Synechococcus</i> sp.", Lipids, 35:10 (2000), pp. 1061-1064.	
	CF**	Magnuson, K. et al., "Regulation of Fatty Acid Biosynthesis in <i>Escherichia coli</i> ", 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 <i>Porphyridium Cruentum</i> ", 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 <i>Phaeodactylum tricornutum</i> Front-End Desaturases Involved in Eicosapentaenoic Acid Biosynthesis", Eur. J. Biochem. 269 (2002), pp. 4105-4113.	
	CM**	Totani, N. et al., "The Filamentous Fungus <i>Mortierella alpina</i> , High in Arachidonic 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**	Tvrdek, 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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	CS**	Sakuradani, E. et al., " Δ 6-Fatty Acid Desaturase from an Arachidonic Acid-Producing <i>Mortierella</i> Fungus Gene Cloning and Its Heterologous Expression in a Fungus, <i>Aspergillus</i> ", Gene 238 (1999), pp. 445-453.	
	CT**	Kinney, A.J., "Genetic Engineering 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 <i>OLE1</i> Gene of <i>Saccharomyces cerevisiae</i> Encodes the Δ 9 Fatty Acid Desaturase and Can Be Functionally Replaced by the Rat Stearoyl-CoA Desaturase Gene", The Journal 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 Δ 12- and Δ 6-Desaturases from <i>Mortierella alpina</i> and Recombinant Production of γ -Linolenic Acid in <i>Saccharomyces cerevisiae</i> ", 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 <i>Shewanella</i> sp. in a Transgenic Marine Cyanobacterium, <i>Synechococcus</i> 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 <i>Brassica napus</i> ", 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 Δ 6-polyunsaturated Fatty Acids from the Moss <i>Physcomitrella patens</i> ", The Plant Journal 31:3 (2002), pp. 255-268.	
	CG1**	Millar, A.A. et al., " <i>CUT1</i> , 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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	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 <i>Phytophthora infestans</i> 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**	Abadi, 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 <i>Phytophthora infestans</i> cDNA, mRNA sequence." Database EMBL, Accession No. BE777235, September 21, 2000.
	CQ1**	Domergue, F. et al., "Cloning and Functional Characterization of <i>Phaeodactylum tricornutum</i> 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 <i>Phytophthora infestans</i> 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 <i>Porphyridium cruentum</i> ", Plant Physiol. 114 (1997), pp. 223-230.
	CT1**	Pereira, S.L. et al., "A Novel ω 3-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 <i>Arabidopsis</i> ", Proc. Natl. Acad. Sci. USA 94 (1997), pp. 1142-1147.
	CW1**	KAJIKAWA, M., et al., "Isolation and Functional Characterization of Fatty Acid Δ 5-Elongase Gene from the Liverwort <i>Marchantia polymorpha</i> L.", FEBS Letters, 2006, Vol. 580, pp. 149-154.
	CX1**	ROBERT, S. S., et al., "Isolation and Characterisation of a Δ 5-Fatty Acid Elongase from the Marine Microalga <i>Pavlova salina</i> ", 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 ω 3-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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CC2**	DOMERGUE, F., et al., "New Insight into <i>Phaeodactylum tricornutum</i> Fatty Acid Metabolism. Cloning and Functional Characterization of Plastidial and Microsomal Δ 12-Fatty Acid Desaturases", <i>Plant Physiology</i> , 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", <i>Nature Biotechnology</i> , 2005, Vol. 23, pp. 1013-1017.
CE2**	NAKAMURA, M. T., et al., "Structure, Function, and Dietary Regulation of Δ 6, Δ 5, and Δ 9 Desaturases", <i>Annu. Rev. Nutr.</i> , 2004, Vol. 24, pp. 345-376.
CF2**	" <i>P. patens</i> Delta6 Elongase SEQ ID 29", <i>GeneSeq Database Accession No. ABG73608</i> , March 25, 2003.
CG2**	"Subname: Full = Polyunsaturated Fatty Acid Elongase elvol5a", <i>UniProt Database Accession No. Q8AWE7</i> , October 25, 2005.
CH2**	"Polyunsaturated Fatty Acid Elongase (ELOVL Family Member 5, Elongation of Long Chain Fatty Acids) (YEAST)", <i>UniProt Database Accession No. Q8AX86</i> , March 1, 2003.
CI2**	"633167 NCCWA 1RT <i>Oncorhynchus Mykiss</i> cDNA Clone 1RT126D03_B_B02 5', mRNA Sequence", <i>EMBL Database Accession No. CA360014</i> , November 7, 2002.
CJ2**	HUANG, Y.-S., et al., "Enzymes for Transgenic Biosynthesis of Long-Chain Polyunsaturated Fatty Acids", <i>Biochimie</i> , 2004, Vol. 86, No. 11, pp. 793-798.
CK2**	" <i>Physcomitrella patens</i> Desaturase Encoding cDNA SEQ ID NO 7", <i>GeneSeq Database Accession No. ABV74260</i> , March 28, 2003.
CL2**	" <i>Phaeodactylum tricornutum</i> Desaturase Encoding cDNA SEQ ID NO 11", <i>GeneSeq Database Accession No. ABV74262</i> , March 28, 2003.
CM2**	Sprecher, H. "Metabolism of Highly Unsaturated n-3 and n-6 Fatty Acids", <i>Biochimica et Biophysica Acta</i> , 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**	" <i>Phaeodactylum tricornutum</i> Elongase Encoding cDNA SEQ ID NO 9", <i>GeneSeq Database Accession No. ABV74261</i> , March 28, 2003.
CP2**	Bork, P., et al., "Go Hunting in Sequence Databases but Watch Out for the Traps", <i>Trends in Genet.</i> 12:10 (1996), pp. 425-427.
CQ2**	Broun, P., et al., "Catalytic Plasticity of Fatty Acid Modification Enzymes Underlying Chemical Diversity of Plant Lipids", <i>Science</i> 282:5392 (1998), pp. 1315-1317.
CR2**	van de Loo, F. J., et al., "An Oleate 12-Hydroxylase from <i>Ricinus communis</i> L. is a Fatty Acyl Desaturase Homolog", <i>Proc. Natl. Acad. Sci. U S A</i> 92:15 (1995), pp. 6743-6747.
CS2**	Doerks, T., et al., "Protein Annotation: Detective Work for Function Prediction", <i>Trends in Genet.</i> 14:6 (1998), pp. 248-250.
CT2**	Brenner, S. E., "Errors in Genome Annotation", <i>Trends in Genet.</i> 15:4 (1999), pp. 132-133.
CU2**	Yu, Z., et al., "Study on Nutritional Function of Polyunsaturated Fatty Acid", <i>China Feed</i> , 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", <i>Nature Biotechnology</i> , 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", <i>Marine Biotechnology</i> , 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.

10 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.

15 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 $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity, where the nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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.

25 In a preferred embodiment there is additionally provision of further nucleic acid sequences which code for a polypeptide having the activity of an $\omega 3$ -desaturase and/or of a $\Delta 4$ -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 $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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 Prokaryotes*. 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 possible 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.

5 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).

10 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 Engineering*, Ed.: JK Setlow, 18:111-13; Gerhardt (1992) *Prog. Lipid R.* 31:397-417; Günemann-Schäfer & Kindl (1995) *Biochim. Biophys Acta* 1256:181-186; Kunau et al. (1995) *Prog. Lipid Res.* 15 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.

20 Depending on the desaturation pattern, two large classes of polyunsaturated fatty acids, the ω 6 and the ω 3 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).

25 The fatty acid linoleic acid ($18:2^{\Delta 9,12}$) acts as starting material for the ω 6 metabolic pathway, while the ω 3 pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed from linoleic acid by the activity of an ω 3-desaturase (Tocher et al. (1998) *Prog. Lipid Res.* 37: 73-117; Domergue et al. (2002) *Eur. J. Biochem.* 269: 4105-4113).

30 Mammals, and thus also humans, have no corresponding desaturase activity (Δ 12- and ω 3-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 ω 6-fatty

acid and the two ω 3-fatty acids eicosapentaenoic acid (= EPA, 20:5^{Δ5,8,11,14,17}) and docosahexaenoic acid (DHA, 22:6^{Δ4,7,10,13,17,19}) are synthesized via a sequence of desaturase and elongase reactions.

5 The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C₂₀- and C₂₂-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 dehydration 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).

10 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 ω 3-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 ω 3-fatty acids to the food (Shimikawa (2001) *World Rev. Nutr. Diet.* 88: 100-108).

20 ω 3-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. ω 6-fatty acids such as arachidonic acid tend to have a negative effect in connection with these rheumatological diseases.

25 ω 3- and ω 6-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 PG₂ series) which are formed from the ω 6-fatty acids, generally promote inflammatory reactions, while eicosanoids (known as the PG₃ series) from ω 3-fatty acids have little or no proinflammatory effect.

Polyunsaturated long-chain ω 3-fatty acids such as eicosapentaenoic acid (= EPA, C20:5 $\Delta^{5,8,11,14,17}$) or docosahexaenoic acid (= DHA, C22:6 $\Delta^{4,7,10,13,16,19}$) 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 ω 3-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 $\Delta^{4,7,10,13,16,19}$) or eicosapentaenoic acid (= EPA, C20:5 $\Delta^{5,8,11,14,17}$) 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 *Cryptocodinium* 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 $\Delta^{5,8,11,14}$), dihomo- γ -linolenic acid (DHGL, C20:3 $\Delta^{8,11,14}$) or docosapentaenoic acid (DPA, C22:5 $\Delta^{7,10,13,16,19}$) 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 Δ 9-desaturase. WO 93/11245 claims a Δ 15-desaturase and WO 94/11516 a Δ 12-desaturase. Further desaturases 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. Δ^6 -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 (C₂₈-C₃₂). 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*, *Porphyridium* species, *Thraustochytrium* species, *Schizochytrium* species or *Cryptocodinium* 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.

- 5 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
- 10 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 Δ 6-desaturases, Δ 6-elongases, Δ 5-desaturases or Δ 4-desaturases. These genes can advantageously be isolated
- 15 from microorganisms and lower plants which produce LCPUFAs and incorporate them in the membranes or triacylglycerides. Thus, it has already been possible to isolate Δ 6-desaturase genes from the moss *Physcomitrella patens* and Δ 6-elongase genes from *P. patens* and from the nematode *C. elegans*.
- 20 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
- 25 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.

- 30 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 $\Delta 5$ -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 $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity,

where the nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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 $\Delta 4$ -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 $\Delta 6$ -desaturase activity, a polypeptide having a $\Delta 6$ -elongase activity, a polypeptide having a $\Delta 5$ -desaturase activity and a polypeptide having a $\Delta 5$ -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 $\Delta 5$ -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
5 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,
10 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
15 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
20 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
25 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
30 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.jp/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelechon (Software Leto) or Geneart (Software GeneOptimizer).

5 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
10 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 $\Delta 5$ -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,
15 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 $\Delta 5$ -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
20 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 $\Delta 5$ -elongase and whose amino acid sequence is modified in one or more positions by comparison with the wild-type
25 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 $\Delta 6$ -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
30 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 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,

5 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

10 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.

15 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 $\Delta 6$ -elongase activity is preferably selected from the group consisting of:

20 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,

25 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,

30 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 $\Delta 6$ -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 $\Delta 5$ -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 $\Delta 4$ -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,
- 5 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
10 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
15 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 Δ 4-desaturase activity is
20 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
25 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
30 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 $\Delta 4$ -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 $\Delta 6$ -desaturase activity, a $\Delta 6$ -elongase activity, a $\Delta 5$ -desaturase activity and a $\Delta 5$ -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 $\Delta 4$ -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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and the $\Delta 5$ -elongase, and if appropriate the $\omega 3$ -desaturase and/or the $\Delta 4$ -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
5 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
10 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-
15 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
20 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.

25 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.
30 (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 $\Delta 5$ -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 $\Delta 5$ -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 $\Delta 4$ -desaturase activity are required. A nucleic acid sequence which codes for a polypeptide having the activity of a $\Delta 4$ -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 $\Delta 5$ -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, monoacylglycerides 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 C₂₀ or C₂₂ 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.

- 5 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 C₁₈-, C₂₀- and/or C₂₂-fatty acid molecules can be isolated in the form of an oil or lipid, for example in the form of compounds such as
10 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
15 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
20 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
25 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
30 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-ynoic acid), crepenynic acid (9-octadecen-12-ynoic acid) 13,14-dihydrooropheic acid, octadecen-

13-ene-9,11-diyonic 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), puniic 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^{Δ3,8,12,15,18,21}).

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, 5 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, 10 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], Mangifera indica (mango) or Anacardium occidentale (cashew), Asteraceae such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, 15 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 annuus (sunflower), Lactuca sativa, Lactuca crispera, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta 20 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, 25 Camelina, Melanosinapis, Sinapis, Arabidopsis, 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), 30 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 sativa (hemp), Convolvulaceae such as the genera Ipomoea, Convolvulus, e.g. the genera and

species *Ipomoea batatas*, *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*
5 *vulgaris* var. *perennis*, *Beta vulgaris* var. *conditiva* or *Beta vulgaris* var. *esculenta* (sugarbeet),
Cucurbitaceae such as the genus *Cucurbita*, 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*
10 *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*,
15 *Cathormion*, *Feuillea*, *Inga*, *Pithecolobium*, *Acacia*, *Mimosa*, *Medicago*, *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*
20 *berterianum*, *Pseudalbizia berteriana*, *Acacia julibrissin*, *Acacia nemu*, *Albizia nemu*,
Feuillea julibrissin, *Mimosa julibrissin*, *Mimosa speciosa*, *Sericanrda julibrissin*, *Acacia*
lebbeck, *Acacia macrophylla*, *Albizia lebbek*, *Feuillea 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*
25 (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*,
30 *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), Lythraeae such as the genus

5 *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.

10 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*,

15 *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*,

20 *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*,

25 *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),

30 *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*
5 *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*,
10 *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*
15 *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,
20 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
25 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,
30 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 viciifolia*), Egyptian clover (*Trifolium alexandrinum*) and Persian clover (*Trifolium resupinatum*). Preferred food plants are for instance lettuce species such as *Lactuca sativa*, *Lactuca crispera*, *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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity, advantageously in combination with nucleic acid sequences which code for polypeptides having $\omega 3$ -desaturase and/or $\Delta 4$ -desaturase activity, and further nucleic acid sequences which code for polypeptides of fatty acid or lipid metabolism, such as further polypeptides having $\Delta 5$ -, $\Delta 6$ -, $\Delta 8$ -, $\Delta 12$ -desaturase or $\Delta 5$ -, $\Delta 6$ - or $\Delta 9$ -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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and/or $\Delta 6$ -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
5 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
10 polypeptide with $\Delta 12$ -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
15 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 $\Delta 12$ -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.

20 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
25 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.

30 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, Pycnococcus, 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 amyliifera*, *Pyramimonas*
5 *disomata*, *Pyramimonas obovata*, *Pyramimonas orientalis*, *Pyramimonas parkae*,
Pyramimonas spinefera, *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*,
10 *Tetraselmis suecica*, *Tetraselmis tetrabrachia*, *Tetraselmis tetrathele*, *Tetraselmis verrucosa*,
Tetraselmis verrucosa fo. *rubens* or *Tetraselmis* sp. or algae from the family Euglenaceae
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*
15 *mixocylindracea*, *Euglena rostrifera*, *Euglena viridis*, *Colacium stentorium*, *Trachelomonas*
cylindrica or *Trachelomonas volvocina*.

Further advantageous plants are algae such as *Isochrysis* or *Cryptocodium*, 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*
20 *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
25 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*
30 *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 *Cryptocodinium*.

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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase and, if appropriate, nucleic acid sequences which code for an $\omega 3$ -desaturase and/or a $\Delta 4$ -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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -elongase activity, advantageously in combination with nucleic acid sequences which encode proteins having $\omega 3$ -desaturase and/or $\Delta 4$ -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, cotyledons, 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 C₂₀ and/or C₂₂ fatty acid molecules having at least four double bonds in the fatty acid molecule, preferably five or six double bonds. These C₂₀ and/or C₂₂ 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), chaulmoogric acid (cyclopentenedodecanoic acid),
5 furan fatty acid (9,12-epoxyoctadeca-9,11-dienonic acid), vernonoic acid (9,10-epoxyoctadec-12-enonic acid), tarinic acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (11-octadecen-9-ynoic acid), 6,9-octadecenynonic acid, pyrulic acid (10-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), puniolic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid) pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballic acid (5,6-octadecadienallenic acid),
15 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 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%
25 based on the total fatty acids and/or no butyric acid, no cholesterol and no nisinic acid (tetracosahexaenoic acid, C23:6^{Δ3,8,12,15,18,21}).

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
30 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 5 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 10 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 15 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 20 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 25 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, H₂SO₄. However, the fatty acids can also be liberated directly without the above-described processing steps.

30 Substrates of the nucleic acid sequences used in the process which encode polypeptides with Δ 6-desaturase, Δ 6-elongase, Δ 5-desaturase and/or Δ 5-elongase activity and optionally nucleic acid sequences which encode polypeptides having ω 3-desaturase and/or Δ 4-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 C₁₆-, C₁₈- or C₂₀-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 C₁₆-fatty acids and/or polyunsaturated C₁₈-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 C₁₆-fatty acids to C₁₈-fatty acids or starting from C₁₈-fatty acids to C₂₀-fatty acids, and after two elongation cycles starting from C₁₆-fatty acids leads to C₂₀-fatty acids. The activity of the desaturases or elongases used in the process according to the invention preferably leads to C₂₀- and/or C₂₂-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 C₂₂-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 C₁₈-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.

5 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 $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, and/or $\omega 3$ -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 $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, and/or $\omega 3$ -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 amplicon 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 protocol (for example

5 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

10 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

15 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

20 *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

25 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 amplicon 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

30 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity and, if appropriate, a $\Delta 4$ -desaturase and/or $\omega 3$ -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 $\omega 3$ -desaturase, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase and/or $\Delta 5$ -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 *Caenorhabditis* 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*, *muscaroides*, *Mortierella*, *Borago*, *Phaeodactylum*, *Cryptocodinium*, 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*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodinium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp., *Muscaroides viallii*, *Mortierella alpina*, *Borago officinalis*, *Phaeodactylum tricorutum*, *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 $\Delta 12$ -desaturase, $\Delta 9$ -elongase or $\Delta 8$ -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 $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 4$ -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 constructs 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase or $\Delta 5$ -elongase and if appropriate the $\omega 3$ -desaturase or $\Delta 4$ -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 inducers, 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, $\Delta 8$ -desaturase, $\Delta 9$ -desaturase, $\Delta 12$ -desaturase and/or $\Delta 9$ -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: 5 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 10 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 15 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 $\Delta 6$ -desaturates, $\Delta 6$ -elongase, $\Delta 5$ -desaturate and/or $\Delta 5$ -elongase genes can be expressed in bacterial cells, insect cells (using baculovirus expression vectors), yeast cells 20 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 25 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 30 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, λ gt11 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 pYeDesaturase1 (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

5 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase or $\Delta 5$ -desaturase and, if appropriate, the $\omega 3$ -desaturase or $\Delta 4$ -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 10 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 15 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 20 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 25 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- 30 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
5 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 $\Delta 5$ -elongase, $\omega 3$ -desaturase, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 4$ -desaturase or $\Delta 5$ -desaturase nucleic acid sequences used, having the sequence SEQ ID NO: 64, SEQ ID
10 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,
15 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,
20 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
25 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
30 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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase and/or Δ 6-elongase nucleic acid sequences disclosed herein by using the
5 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
10 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
15 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
20 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,
25 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
30 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
5 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
10 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
15 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
20 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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase and/or Δ 6-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
25 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
30 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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-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 ω 3-desaturase-, Δ 6-desaturase-, Δ 5-desaturase-, Δ 5-elongase-, Δ 4-desaturase- or Δ 6-elongase-encoding sequence, e.g. by saturation mutagenesis, and the resulting mutants can be screened for the ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-elongase activity described herein in order to identify mutants which have retained the ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-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
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German application number 102006008030.0	this subsequent application	
1	64	<i>Ostreococcus tauri</i>
2	65	<i>Ostreococcus tauri</i>
3	1	<i>Phytium irregulare</i>
4	2	<i>Phytium irregulare</i>
5	171	<i>Traustochytrium</i> sp.
6	172	<i>Traustochytrium</i> sp.
7	51	<i>Thraustochytrium</i> ssp.
8	52	<i>Thraustochytrium</i> ssp.
9	193	<i>Phytophthora infestans</i>
10	194	<i>Phytophthora infestans</i>
11	77	<i>Traustochytrium</i> sp.
12	78	<i>Traustochytrium</i> sp.
13	109	<i>Ostreococcus tauri</i>
n.a.	110	<i>Ostreococcus tauri</i>
14	122	<i>Ostreococcus tauri</i>
n.a.	123	<i>Ostreococcus tauri</i>
15	143	<i>Ostreococcus tauri</i>
16	144	<i>Ostreococcus tauri</i>
17	161	Cauliflower mosaic virus
18	162	Cauliflower mosaic virus
19	163	<i>Thalassiosira pseudonana</i>
20	164	<i>Thalassiosira pseudonana</i>

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.

5 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 $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity. These are the following sequences:

Gene name	SEQ ID	Amino acids
OtELO1.1, ($\Delta 6$ -Elongase)	SEQ ID NO: 143	292
OtELO2.1, ($\Delta 5$ -Elongase)	SEQ ID NO: 109	300

10 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:

15 40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down and resuspended in 100 μ l 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
 20 was carried out in each case with 1 μ l of thawed cells, 200 μM dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 μ l. 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.

25 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 ($\Delta 6$ -elongase) and SEQ ID NO: 109 (coding for a protein identified by SEQ ID NO: 110) ($\Delta 5$ -elongase) were adapted to the codon usage in oilseed rape, flax and soybean. For this purpose, the amino acid sequence of the $\Delta 6$ -elongase and of the $\Delta 5$ -elongase (SEQ ID NO: 144 for the $\Delta 6$ -elongase; SEQ ID NO: 65 for the $\Delta 5$ -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 ($\Delta 5$ -elongase) and SEQ ID NO: 122 (coding for a protein identified by SEQ ID NO: 123) ($\Delta 6$ -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 $\Delta 6$ -elongase sequence) and pOTE2.2 (comprising the $\Delta 5$ -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, ($\Delta 6$ -elongase)	SEQ ID NO: 143	292
pOTE1.2, ($\Delta 6$ -elongase)	SEQ ID NO: 122	292, codon-optimized
pOTE2.1, ($\Delta 5$ -elongase)	SEQ ID NO: 109	300
pOTE2.2, ($\Delta 5$ -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 $\Delta 6$ -elongase and $\Delta 5$ -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 OD₆₀₀ 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 NaHCO₃, 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 NaHCO₃, pH 8.0 and with 2 ml of distilled water. The PE phases were then dried with Na₂SO₄, evaporated under argon and taken up in 100 μ l of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 μ m, 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
5 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
10 the sequence of the $\Delta 5$ -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 conditions described apply to all subsequent experiments unless described otherwise.

15 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.

20 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': gaattcggcgcgccgagctcctcgagcaacgggtccggcggtatagagttgggtaattcga (SEQ ID NO: 200)

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

25

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 μ l of 2mM dNTP

30 1.25 μ l of each primer (10 pmol/ μ l)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

5 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
10 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
15 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:

20 OCS_C 5': aggcctccatggcctgcttaatgagatatgagagacgcc (SEQ ID NO: 202)

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

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

25 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

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

5 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 *Stu*I and then for 12 hours at 25°C with the restriction enzyme *Sma*I. The vector pUC19-Cn11-C was
10 incubated for 12 hours at 25°C with the restriction enzyme *Sma*I. 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
15 product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cn11-C_OCS was verified by sequencing.

In the next step, the Cn11-B promoter was amplified by PCR by means of the following primers:

Cn11-B 5': aggcctcaacggttccggcggtatag (SEQ ID NO: 204)

Cn11-B 3': cccggggttaacgctagcgggcccgatatcggatcccatttttggtggtgattggttct (SEQ ID NO: 205)

20

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

25 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

5

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-Cn11-C_Cn11B_OCS was verified by sequencing.

10 In a further step, the OCS terminator for Cn11B was inserted. To this end, the PCR was carried out using the following primers:

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

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

Composition of the PCR mix (50 µl):

20 5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

25

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
5 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.
10 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': aggcctcaacgggtccggcggtatagag (SEQ ID NO: 208)

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

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

20 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:

25 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 *Stu*I. The vector pUC19-Cnl1-C was incubated for 12 hours at 25°C with the restriction enzyme *Sma*I. 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.

10 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': ggctctgctttaatgagatatgcca (SEQ ID NO: 210)

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

15 Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

20 0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

25 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.
- 10 In the next step, the plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the $\Delta 6$ -, $\Delta 5$ -desaturase and $\Delta 6$ -elongase. To this end, the *Phytium irregulare* $\Delta 6$ -desaturase (WO02/26946) was amplified using the following PCR primers:

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

- 15 D6Des(Pir) 3': ccatgccccgggttacatcgctgggaactcggat (SEQ ID NO: 213)

Composition of the PCR mix (50 μ l):

- 5.00 μ l template cDNA
5.00 μ l 10x buffer (Advantage polymerase) + 25mM $MgCl_2$
20 5.00 μ l of 2mM dNTP
1.25 μ l of each primer (10 pmol/ μ l)
0.50 μ l of Advantage polymerase (Clontech)

PCR reaction conditions:

- 25 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/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/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. $\Delta 5$ -desaturase (WO02/26946). To this end, the *Thraustochytrium* ssp. $\Delta 5$ -desaturase was amplified using the following PCR primers:

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

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

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l 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 *EcoRV*. The vector pUC19-Cnl1_d6Des(Pir) was incubated for 2 hours at 37°C with the restriction enzyme BamHI and
5 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-
10 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)

15 D6Elo(Pp) 3': gcaaaaggagctaaaactgagtgatctaga (SEQ ID NO: 217)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

20 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:

25 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': gtcgatcaacgggtccggcggtatagagttg (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 MgCl₂

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:

5 Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

10 The PCR product was incubated for 2 hours at 37°C with the restriction enzyme Sall. The vector pUC19 was incubated for 2 hours at 37°C with the restriction enzyme Sall. 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
15 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)

20 D12Des(Co) 3': ccatggttaaactcttattacgatacc (SEQ ID NO: 221)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

25 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

5 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.

15 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 Sall. 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 *AscI*. 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
5 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
10 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
15 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
20 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
25 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
30 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 O₁ELO2.2 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 metabolites 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.

5 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
10 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
15 means of GC-MS.

Example 9: Use of the optimized $\Delta 5$ -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
20 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 Sall/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
25 terminator ('triple ligation'). The resulting plasmid pUC19-35S was then employed for cloning PUFA genes. In parallel, the open reading frames of the $\Delta 6$ -desaturase (SEQ ID NO: 1), of the $\Delta 5$ -desaturase (SEQ ID NO: 51) and $\Delta 6$ -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-
30 35S_D6D5E6(Tc). For this purpose, the vector pGPTV was digested with the enzyme Sall, the plasmid pUC-D6 was digested with Sall/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6 was then digested with Sall, 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.

- 5 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 ω 3Des (SEQ ID NO: 193) was cloned into pUC19-35S. The resulting plasmid pUC- ω 3Pi was transferred via SalI/XhoI into the binary
10 vectors pGPTV-D6D5E6(Tc) and pGPTV-D6D5E6(Tp). The resulting vectors pGPTV-D6D5E6(Tc) ω 3Pi and pGPTV-D6D5E6(Tp) ω 3Pi were employed for the plant transformation.

In a further procedure, the open reading frame of the optimized Δ 5-elongase from *Ostreococcus tauri* (SEQ ID NO: 64) and the open reading frame of the Δ 4-desaturase from
15 *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) ω 3Pi. The resulting vector pGPTV-D6D5E6(Tp) ω 3PiE5D4 was employed for the plant transformation.

All the binary vectors were transformed into *E. coli* DH5 α cells (Invitrogen) in accordance
20 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)
25 *Plant Cell Reports* 8:238-242)

The binary vector pGPTV-D6D5E6(Tp) ω 3PiE5D4 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)
30 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 cm²) 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 *Orychopragmus 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 *Orychopragmus violaceus*. Petioles or hypocotyls of freshly germinated sterile plants (each about 1 cm²) 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 $\Delta 6$ -elongase activity or $\Delta 5$ - or $\Delta 6$ -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) ω 3PiE5D4 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) ω 3PiE5D4 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 $\Delta 5$ -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% CaCl₂ (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: $sn-1 + sn-3 = (TAG \times 3 - MAG)/2$.

This positional 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 N₂ and resuspended in 0.5 ml of borate buffer (0.5M, pH 7.5, containing 0.4 mM CaCl₂). 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.

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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 <i>Ostreococcus tauri</i> 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 <i>Orychophragmus violaceus</i>											
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 <i>Arabidopsis thaliana</i>											
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-7	18:2n-9	18:2n-6	18:3n-6	18:3n-2	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		82.03							
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	24.98	2.22	4.16
sn-2	1.62	0.64	9.33	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	4.89	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	4.58	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	264	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	0.03	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

DEDG	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	55.06	0.27	0.00	0.00	0.35	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	16:0	18:1	18:2	γ 18:3	α 18:3	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	4.3	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
5 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
10 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.
- 15 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
 - 20 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:
 - 25 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.
- 5 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
10 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 ω 3-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
15 oils, lipids and/or fatty acids comprise a total amount of at least 54% by weight of polyunsaturated ω 3-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.
- 20 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
25 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
30 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
5 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
10 expressing in a Brassica plant a nucleic acid encoding a $\Delta 6$ -desaturase, a nucleic acid encoding a $\Delta 5$ -desaturase, a nucleic acid encoding a $\Delta 6$ -elongase, a nucleic acid encoding a $\omega 3$ -desaturase, a nucleic acid encoding a $\Delta 5$ -elongase, and a nucleic acid encoding a $\Delta 4$ -desaturase, wherein said nucleic acid encoding a $\Delta 5$ -elongase is codon-optimized by adapting to the codon usage of Brassica.

15 18. The method of claim 17, wherein said nucleic acid encoding a $\Delta 5$ -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%,
20 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
25 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 $\Delta 5$ -elongase comprises a translated section coding for a protein having at least 95%, 96%, 97%, 98%, 99% or 100%
30 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 polypeptide with a $\Delta 6$ desaturase activity, at least one nucleic acid sequence coding for a polypeptide with a $\Delta 6$ elongase activity, at least one nucleic acid sequence coding for a polypeptide with a $\Delta 5$ desaturase activity, and at least one nucleic acid sequence coding for a polypeptide with a $\Delta 5$ elongase activity, the nucleic acid sequence coding for a polypeptide with a $\Delta 5$ 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 $\Delta 4$ 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.)
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Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	24984	no	7
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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:					
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6	Information Disclosure Statement (IDS) Form (SB08)	SB08.pdf	80562	no	6
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Total Files Size (in bytes):			1940187		

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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.

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.

Electronic Acknowledgement Receipt

EFS ID:	26838159
Application Number:	15256914
International Application Number:	
Confirmation Number:	4050
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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
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Payment was successfully received in RAM	\$1760
RAM confirmation Number	090616INTEFSW13005900
Deposit Account	500573
Authorized User	Jamie Jensen-Smith

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	24984	no	7
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Information:					
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Total Files Size (in bytes):			1940187		

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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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Application No: 15256914 Version No: 1.0

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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

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 Qiu, Xiao
 Wu, Guohai
 Cheng, Bifang
 Truksa, Martin
 Wetjen, Tom

<120> METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

<130> 074017-0013-01-US

<140> US 15/256,914

<141> 2016-09-06

<150> 12/280,090

<151> 2008-08-20

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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			
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Arg Arg Leu Arg Val Lys Val Lys Gly Met Gly Leu Tyr Asp Ala Ser			
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Val Ala Gly Val Ile Met Gly Leu Phe Tyr Gln Gln Ser Gly Trp Leu			
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180	185	190	
Asn Leu Ile Gly Cys Leu Val Gly Asn Ala Trp Gln Gly Phe Ser Met			
195	200	205	
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 Tyr Val Phe Thr Glu Phe Ser Phe Gly Ile Phe Asp Lys Val Glu Phe
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 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
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 His Pro Gly Gly Ala Gln Val Leu Leu Thr His Val Gly Lys Asp Ala
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Gly His His Gln Cys Phe Glu Asp Arg Thr Trp Asn Asp Va



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Table with 7 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY.DOCKET.NO, TOT CLAIMS, IND CLAIMS. Row 1: 15/256,914, 09/06/2016, 1629, 1760, 074017-0013-01-US, 22, 2

CONFIRMATION NO. 4050

FILING RECEIPT

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



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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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
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).

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Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

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APPLICATION AS FILED - PART I			SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
	(Column 1)	(Column 2)					
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)
BASIC FEE <small>(37 CFR 1.16(a), (b), or (c))</small>	N/A	N/A	N/A			N/A	280
SEARCH FEE <small>(37 CFR 1.16(k), (l), or (m))</small>	N/A	N/A	N/A			N/A	600
EXAMINATION FEE <small>(37 CFR 1.16(o), (p), or (q))</small>	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS <small>(37 CFR 1.16(j))</small>	22	minus 20 = *				x 80 =	160
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>	2	minus 3 = *				x 420 =	0.00
APPLICATION SIZE FEE <small>(37 CFR 1.16(s))</small>	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						SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
AMENDMENT A		(Column 1)	(Column 2)	(Column 3)						
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	x	=	OR	x	=
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	x	=	OR	x	=
Application Size Fee (37 CFR 1.16(s))								OR		
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								OR		
TOTAL ADD'L FEE								OR	TOTAL ADD'L FEE	
AMENDMENT B		(Column 1)	(Column 2)	(Column 3)						
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	x	=	OR	x	=
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	x	=	OR	x	=
Application Size Fee (37 CFR 1.16(s))								OR		
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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Table with 4 columns: APPLICATION NUMBER (15/256,914), FILING OR 371(C) DATE (09/06/2016), FIRST NAMED APPLICANT (Petra Cirpus), ATTY. DOCKET NO./TITLE (074017-0013-01-US)

CONFIRMATION NO. 4050

INFORMAL NOTICE



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

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/



Bescheinigung

Certificate

Attestation

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

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

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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 Président de l'Office européen des brevets
p.o.

R.C. van Dijk



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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
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Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren

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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
5 der Pflanze bereitgestellt werden mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 6$ -Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Desaturase-Aktivität kodiert; und mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Elongase-Aktivität kodiert,
10 wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -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.

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In einer bevorzugten Ausführungsform werden zusätzlich weitere Nukleinsäuresequenzen, die für ein Polypeptid mit der Aktivität einer $\omega 3$ -Desaturase und/oder einer $\Delta 4$ -Desaturase kodieren, in der Pflanze bereitgestellt.

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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.

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Die Erfindung betrifft weiterhin rekombinante Nukleinsäuremoleküle, umfassend mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -Desaturase-Aktivität
30 kodiert; mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 5$ -

Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert; und mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 5$ -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 Prokaryotes*. 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.

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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).

10

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 Engineering, 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 Engineering, 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.

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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.

30

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 Desaturaseaktivität (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 C₂₀- bzw. C₂₂-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).

5

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.

10

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

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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. PG₂-Serie), die aus ω -6-Fettsäuren gebildet werden, fördern in der Regel Entzündungsreaktionen, während Eicosanoide (sog. PG₃-Serie) aus ω -3-

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Fettsäuren geringe oder keine entzündungsfördernde Wirkung haben.

Mehrfach ungesättigte langkettige ω -3-Fettsäuren wie Eicosapentaensäure (= EPA, C₂₀:5 Δ ^{5,8,11,14,17}) oder Docosahexaensäure (= DHA, C₂₂:6 Δ ^{4,7,10,13,16,19}) sind wichtige

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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).

30

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.

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Hauptsächlich werden die verschiedenen Fettsäuren und Triglyceride aus Mikroorganismen wie *Mortierella* oder *Schizochytrium* oder aus Öl-produzierenden Pflanzen wie Soja, Raps und Algen wie *Cryptocodinium* oder *Phaeodactylum* und weiteren gewonnen, wobei sie in der Regel in Form ihrer Triacylglyceride (= Triglyceride = Tri-
10 glycerole) 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
15 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
20 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
25 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
30 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 (C₂₈-C₃₂). 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, Porphyridium-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 Mutantstämmen 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 $\Delta 5$ -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 $\Delta 6$ -Desaturase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Desaturase-Aktivität kodiert; und mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Elongase-Aktivität kodiert, wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -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 $\Delta 4$ -Desaturase-Aktivität kodiert, in der Pflanze bereitgestellt werden.

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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 $\Delta 6$ -Desaturase-Aktivität, ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität, ein Polypeptid mit einer $\Delta 5$ -Desaturase-Aktivität und ein Polypeptid mit einer $\Delta 5$ -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.

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Die Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 5$ -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.

20

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

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30

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
5 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 Expressi-
on erhöhen. Ohne an eine Hypothese gebunden sein zu wollen, wird angenommen, dass
die kodonoptimierten DNA-Sequenzen eine effizientere Translation ermöglichen und die
10 daraus gebildeten mRNAs möglicherweise eine höhere Halbwertszeit in der Zelle besit-
zen 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 Organis-
mus, aus dem die Nukleinsäuresequenz ursprünglich stammt.

15

Für viele Organismen, von denen die DNA-Sequenz einer größeren Zahl von Genen be-
kannt ist, gibt es Tabellen, denen man die Häufigkeit der Verwendung bestimmter Ko-
dons in dem jeweiligen Organismus entnehmen kann. Mit Hilfe dieser Tabellen lassen
sich Proteinsequenzen mit relativ großer Genauigkeit in eine DNA-Sequenz zurück-
20 übersetzen, 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
25 Geneart (Software GeneOptimizer).

Die Anpassung der Sequenzen an die Kodonverwendung in einem bestimmten Orga-
nismus kann unter Zuhilfenahme verschiedener Kriterien erfolgen. Zum einen kann für
eine bestimmte Aminosäure immer das am häufigsten im ausgewählten Organismus
30 vorkommende Kodon verwendet werden, zum anderen kann aber auch die natürliche
Frequenz der verschiedenen Kodons berücksichtigt werden, so dass alle Kodons für ei-
ne 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
5 Kodons ebenfalls geeignet ist.

Besonders bevorzugt ist eine Nukleinsäuresequenz aus *Ostreococcus tauri*, die für ein Polypeptid mit einer $\Delta 5$ -Elongase-Aktivität kodiert, wie beispielsweise das in Seq ID No. 110 dargestellte Polypeptid, zumindest an die Kodonverwendung in Raps, Soja
10 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 $\Delta 5$ -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
15 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.

20 Es versteht sich, dass auch solche kodonoptimierten DNA-Sequenzen von der Erfindung erfasst sind, die für ein Polypeptid mit der Aktivität einer $\Delta 5$ -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.

25 Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -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

30 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
5 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 zur der in Seq ID No. 1 angegebenen Sequenz zu mindestens 60%, 65%, 70%, 75% oder 80%, bevorzugt zu mindestens 81%, 82%, 83%, 84%,
10 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.

15 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.

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

25 Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -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,

30 b) Nukleinsäuresequenzen, die für die in Seq ID No. 172, 174,176,178,180,182 oder 184, 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 oder 192 angegebenen Aminosäurepattern aufweisen.
- Insbesondere handelt es sich bei der Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert ebenfalls um eine nach der vorliegenden Erfindung kodonoptimierten Sequenz, bevorzugt um die in der SEQ ID NO: 122 dargestellte Nukleinsäuresequenz.
- Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 5$ -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

- 5 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 angegebenen Aminosäurepattern aufweisen.

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

10

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 Δ 4-Desaturase kodieren, in die Pflanze eingebracht.

- 15 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,
- 20 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
- 25 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.

- 30 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.

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Bevorzugt ist die Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 4$ -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,
- 10 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,
- 15 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%,
- 20 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 angegebenen Aminosäurepattern aufweisen.

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Die $\Delta 4$ -Desaturase, die im erfindungsgemäßen Verfahren vorteilhaft verwendet wird, katalysiert die Einführung einer Doppelbindung in die Fettsäure Docosapentaensäure, was zur Bildung von Docosahexaensäure führt.

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Für das erfindungsgemäße beschriebene Verfahren ist es vorteilhaft, in die Pflanzen zusätzlich zu den Nukleinsäuresequenzen, die für Polypeptide mit einer $\Delta 6$ -Desaturase-Aktivität, einer $\Delta 6$ -Elongase-Aktivität, einer $\Delta 5$ -Desaturase-Aktivität und einer $\Delta 5$ -

Elongase-Aktivität kodieren, sowie den ggf. eingebrachten Nukleinsäuresequenzen, die für ein Polypeptid mit einer ω -3-Desaturase-Aktivität und/oder einer Δ 4-Desaturase-Aktivität kodieren, zusätzlich weitere Nukleinsäuren einzubringen, die für Enzyme des Fettsäure- oder Lipidstoffwechsels kodieren.

5

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)-

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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-Acetylasen, Lipoxygenasen, Triacylglycerol-Lipasen, Allenoxid-Synthasen, Hydroperoxid-Lyasen oder Fettsäure-Elongase(n) in Kombination

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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

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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

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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.

10 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.

15 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.

- 5 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.
- 10 "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
- 15 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 be-

20 kannten 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

25 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

30 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.

5 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
10 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.-%, be-
15 vorzugt 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
20 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 Δ 4-Desaturase-Aktivität kodiert, benötigt werden. Vorteilhaft wird eine Nukleinsäuresequenz, die für ein Polypeptid mit der Aktivität einer Δ 4-Desaturase-Aktivität kodiert, ausgewählt
25 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
30 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 Kartoffel-

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

5 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.

10 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.

15 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
20 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
25 bis 12 C-Atomen oder langkettige Fettsäuren mit 14 bis 24 C-Atomen. Bevorzugt enthalten sie langkettige Fettsäuren, besonders bevorzugt C₂₀- oder C₂₂-Fettsäuren.

30 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 C₁₈-, C₂₀- und/oder C₂₂-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-diyonsäure, 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^{Δ3,8,12,15,18,21}).

5

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.

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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.

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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, E-laeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Fagaceae, Grossulariaceae, Juglandaceae, Lauraceae, Liliaceae, Linaceae, Malvaceae, Moraceae, Musaceae, Oleaceae, Oxalida-

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

Beispielhaft seien die folgenden Pflanzen genannt: Anacardiaceae wie die Gattungen
5 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 (Korn-
10 blume), Cichorium intybus (Wegwarte), Cynara scolymus (Artischocke), Helianthus an-
nus (Sonnenblume), Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola
L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lac-
tuca sativa subsp. romana, Locusta communis, Valeriana locusta (Salat), Tagetes lucida,
Tagetes erecta oder Tagetes tenuifolia (Studentenblume), Apiaceae wie die Gattung
15 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 (Hasel-
nuss), Boraginaceae wie die Gattung Borago z.B. die Gattung und Art Borago officinalis
(Borretsch), Brassicaceae wie die Gattungen Brassica, Camelina, Melanosinapis, Sina-
pis, Arabidopsis z.B. die Gattungen und Arten Brassica napus, Brassica rapa ssp.
20 (Raps), Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea
var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Came-
lina sativa, Melanosinapis communis (Senf), Brassica oleracea (Futterrübe) oder Arabi-
dopsis thaliana, Bromeliaceae wie die Gattungen Anana, Bromelia (Ananas) z.B. die
Gattungen und Arten Anana comosus, Ananas ananas oder Bromelia comosa (Ananas),
25 Caricaceae wie die Gattung Carica wie die Gattung und Art Carica papaya (Papaya),
Cannabaceae wie die Gattung Cannabis wie die Gattung und Art Cannabis sativa (Hanf),
Convolvulaceae wie die Gattungen Ipomoea, Convolvulus z.B. die Gattungen und Arten
Ipomoea batatas, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipo-
moea fastigiata, Ipomoea tiliacea, Ipomoea triloba oder Convolvulus panduratus (Süß-
30 kartoffel, Batate), Chenopodiaceae wie die Gattung Beta wie die Gattungen und Arten
Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var. vulgaris, Beta maritima, Be-
ta vulgaris var. perennis, Beta vulgaris var. conditiva oder Beta vulgaris var. esculenta

(Zuckerrübe), Cucurbitaceae wie die Gattung Cucurbita 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, Medicago, 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, Pseudalbizia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuillea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbeck, Feuillea 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), *Lythraeae* 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 *Chrootheca*, *Flintiella*, *Petrovanella*, *Porphyridium*, *Rhodella*, *Rhodorus*, 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 *Coffea* 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).
- 20 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.

5 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 crisper*, *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*.

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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 ^{Δ 9,12}) vor, so können als Produkte des Verfahrens nur GLA, DGLA und ARA ent-

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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 ^{Δ 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 E-laeagnaceae 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 longifillia*, *Mamiella gilva*, *Mantoniella squamata*, *Micromonas pusilla*, *Nephroselmis olivacea*, *Nephroselmis pyriformis*, *Nephroselmis rotunda*, *Ostreococcus tauri*, *Ostreococcus* sp., *Prasinocladus ascus*, *Prasinocladus lubricus*, *Pycnococcus provasolii*, *Pyramimonas amyliifera*, *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/

- 5 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,
- 10 *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*.

- 25 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
- 30 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 zustande gekommenen 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

5 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.

15 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.

25

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-

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

- 5 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
10 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
15 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.
20

- Transgene Pflanzen, die die im erfindungsgemäßen Verfahren synthetisierten mehrfach ungesättigten, lange-kettigen 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.
25

- 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
30

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

- 5 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
10 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
15 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
20 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 entfer-
25 nen, 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 C₂₀-
30 und/oder C₂₂-Fettsäuremoleküle mit mindestens vier Doppelbindungen im Fettsäuremolekül, vorzugsweise fünf oder sechs Doppelbindungen. Diese C₂₀- und/oder C₂₂-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
5 % 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.

10 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
15 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
20 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-diyonsäure, Petroselensäure (cis-6-Octadecenonsäure), 9c,12t-Octadecadiensäure, Calendulasäure (8t10t12c-Octadecatriensäure), Catalpinsäure (9t11t13c-Octadecatriensäure), Eleosterinsäure (9c11t13t-Octadecatriensäure), Jacarinsäure
25 (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,
5 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.

10 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
15 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
20 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. H₂SO₄. Die Freisetzung der Fettsäuren kann
25 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
30 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 C₁₆-, C₁₈- oder C₂₀-Fettsäuren. Bevorzugt werden die im Verfahren als Substrate umgesetzten Fettsäuren in Form ihrer Acyl-CoA-Ester und/oder ihrer Phospholipid-Ester umgesetzt.

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Zur Herstellung der erfindungsgemäßen langkettigen PUFAs müssen die gesättigten, einfach ungesättigten C₁₆-Fettsäuren und/oder mehrfach ungesättigten C₁₈-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 C₁₆-Fettsäuren zu C₁₈-Fettsäuren oder ausgehend von C₁₈-Fettsäuren zu C₂₀-Fettsäuren, und nach zwei Elongationsrunden ausgehend von C₁₆-Fettsäuren zu C₂₀-Fettsäuren. Die Aktivität der im erfindungsgemäßen Verfahren verwendeten Desaturasen und Elongasen führt vorzugsweise zu C₂₀- und/oder C₂₂-Fettsäuren vorteilhaft mit mindestens zwei oder drei Doppelbindungen im Fettsäuremolekül, vorzugsweise mit vier, fünf oder sechs Doppelbindungen, besonders bevorzugt zu C₂₂-Fettsäuren mit mindestens fünf Doppelbindungen im Fettsäuremolekül. Besonders bevorzugt als Produkte des erfindungsgemäßen Verfahrens sind Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure. Die C₁₈-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.

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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

5 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

10 der Pool an veresterten mehrfach ungesättigten Fettsäuren in den transgenen Pflanzen erhöht, vorteilhaft in Form der Phosphatidylester und/oder Triacyl ester.

Die im erfindungsgemäßen Verfahren verwendeten Sequenzen werden einzeln in Ex-

15 pressionskonstrukte 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.

20 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

25 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,

30 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 Agrobacterium-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.

5 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.

20 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-Paket (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.

- 5 Vorteilhaft im Verfahren verwendbare Nucleinsäuren 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*, *Cryptocodinium*, speziell
- 10 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*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodinium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium sp.*, *Muscarioides viallii*, *Mortierella alpina*, *Borago*
- 15 *officinalis*, *Phaeodactylum tricornutum*, *Caenorhabditis elegans* oder besonders vorteilhaft aus *Pytium irregulare*, *Thraustochytrium sp.* und/oder *Ostreococcus tauri*.

Im erfindungsgemäßen Verfahren können zusätzlich Nucleotidsequenzen verwendet werden, die für eine Δ -12-Desaturase, Δ -9-Elongase oder Δ -8-Desaturase kodieren. Die

20 im Verfahren verwendeten Nucleinsäuresequenzen werden vorteilhaft in einer Expressionskassette, die die Expression der Nucleinsäuren in Pflanzen ermöglicht, eingebracht.

Die Nucleinsäuresequenzen, 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

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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.

5 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 unter-
10 schiedlich 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
15 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.
20 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
25 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.

30 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 Agrobacterium 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

5 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

10 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

15 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

20 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

25 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

30 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 Biotechnology, 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.

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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*

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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
5 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-
10 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.

15 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
20 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
25 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,
30 pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λ gt11 or pBdCl, 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 Octopinsynthese 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 Kermodé (1996) Crit. Rev. Plant Sci. 15 (4): 285-423 und darin zitierte Literaturstellen).

10 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 Nucleinsäuren (z.B. DNA) in eine Wirtszelle, einschließlich Calciumphosphat- oder Calciumchlorid-Kopräzipitation, DEAE-Dextran-vermittelte Transfektion, Lipofektion, natürliche Kompetenz, chemisch vermittelter Transfer, Elektroporation oder Teilchenbeschuss, 15 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 20 Methods in Molecular Biology, 1995, Bd. 44, Agrobacterium protocols, Hrsgb: Gartland und Davey, Humana Press, Totowa, New Jersey.

Der Begriff "Nucleinsäure(molekül)", wie hier verwendet, umfasst in einer vorteilhaften 25 Ausführungsform zudem die am 3'- und am 5'-Ende des kodierenden Genbereichs gelegene untranslatierte Sequenz: mindestens 500, bevorzugt 200, besonders bevorzugt 100 Nucleotide der Sequenz stromaufwärts des 5'-Endes des kodierenden Bereichs und mindestens 100, bevorzugt 50, besonders bevorzugt 20 Nucleotide der Sequenz stromabwärts des 3'-Endes des kodierenden Genbereichs. Ein "isoliertes" Nucleinsäuremolekül wird von anderen Nucleinsäuremolekülen abgetrennt, die in der natürlichen Quelle 30 der Nucleinsäure vorliegen. Eine "isolierte" Nucleinsäure hat vorzugsweise keine Sequenzen, welche die Nucleinsäure 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 verwendete isolierte Δ -6-Desaturase-, Δ -6-Elongase- oder Δ -5-Desaturase- sowie ggf. das ω -3-Desaturase- oder Δ -4-Desaturase-Molekül zum Beispiel
5 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
10 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
15 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
20 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-
25 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,
30 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 Nucleinsäure kann unter Verwendung von cDNA oder alternativ von genomischer DNA als Matrize und geeigneten Oligonucleotidprimern nach Standard-PCR-Amplifikationstechniken amplifiziert werden. Die so amplifizierte Nucleinsäure kann in einen geeigneten Vektor kloniert werden und mittels DNA-Sequenzanalyse charakterisiert werden. Oligonucleotide 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-Nucleinsäuresequenzen 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 Nucleotidsequenzen oder ihren Homologen, Derivaten oder Analoga oder Teilen davon. Weiterhin sind isolierte Nucleinsäuremoleküle einer Nucleotidsequenz 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 Nucleotidsequenzen 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 Nucleotiden 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.

- 5 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.
- 20 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.
- 25 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 Waschsritten 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
- 30 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-
5 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.
10 171, 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-
15 vermittelte Mutagenese, eingebracht werden. Vorzugsweise werden konservative Ami-
nosäuresubstitutionen 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
20 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,
25 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 Seiten-
kettenfamilie ausgetauscht. Alternativ können bei einer anderen Ausführungsform die
30 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 kodie-
renden Sequenz eingebracht werden, z.B. durch Sättigungsmutagenese, und die resul-

5 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.

10 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.

15 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.

20 Konkordanztable 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.

65

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

5

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.

10

Beispiel 2: Sequenzanalyse rekombinanter DNA

15

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.

5 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:

10

Gen-Name	SEQ ID	Aminosäuren
OtELO1.1, (Δ -6-Elongase)	SEQ ID NO. 143	292
OtELO2.1, (Δ -5-Elongase)	SEQ ID NO. 109	300

15

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:

20

40 ml einer *Ostreococcus tauri* Kultur in der stationären Phase wurden abzentrifugiert und in 100 μ l 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 μ l aufgetauten Zellen, 200 μ M 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.

5

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 ($\Delta 6$ -Elongase) und Seq ID No. 109 (kodierend für ein durch die Seq ID No. 110 gekennzeichnetes Protein)($\Delta 5$ -Elongase) an die Kodonverwendung in Raps, Lein und Soja angepasst. Dazu wurde die Aminosäuresequenz der $\Delta 6$ -Elongase und der $\Delta 5$ -Elongase (SEQ ID NO. 144 für die $\Delta 6$ -Elongase; SEQ ID NO. 65 für die $\Delta 5$ -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 ($\Delta 5$ -Elongase) und SEQ ID NO. 122 (kodierend für ein durch die SEQ ID NO. 123 gekennzeichnetes Protein)($\Delta 6$ -Elongase) angegeben sind, wurden in vitro synthetisiert.

20

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 $\Delta 6$ -Elongase-Sequenz) und pOTE2.2 (enthaltend die $\Delta 5$ -Elongase-Sequenz) erhalten wurden.

25

Übersicht zu den in den Hefe-Vektor pYES2.1/V5-His-TOPO klonierten Elongase-Sequenzen:

30

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 Δ 6-Elongase bzw. Δ 5-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 Komplet-Minimalmedium (CMdum)-Agarplatten mit 2% Glucose, aber ohne Uracil. Nach der Selektion wurden je drei Transformanten zur weiteren funktionellen Expression ausgewählt.

Für die Expression 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 OD₆₀₀ von 0,05 angeimpft. Dabei wurde der Hefekultur, die mit pOTE1.1 und pOTE1.2 transformiert 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

5 Hefen, die wie in Beispiel 5 mit den Plasmiden pYES2, pOTE1.2, und pOTE2.1 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 NaHCO₃, 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 NaHCO₃, pH 8,0 und mit 2 ml Aqua dest. gewaschen. Anschließend wurden die PE-Phasen mit Na₂SO₄ 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.

30 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 $\Delta 5$ -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

10

Die folgenden beschriebenen allgemeinen Bedingungen gelten für alle nachfolgenden Versuche, wenn nicht anders beschrieben.

15

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.

20

Ausgangspunkt der Klonierung war der Klonierungsvektor pUC19 (Maniatis et al.). Im ersten Schritt wurde das Conlinin-Promotor-Fragment mit folgenden Primern amplifiziert:

25

Cnl1 C 5': gaattcggcgcgccgagctcctcgagcaacgggtccggcggtatagagttgggtaattcga

Cnl1 C 3': cccgggatcgatgccggcagatctccaccatttttggtggtgat

Zusammensetzung des PCR-Ansatzes (50 μ l):

5,00 μ l Template cDNA

30

5,00 μ l 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 μ l 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

5

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

10

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.

15

20

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

25

OCS_C 3': cccgggccggacaatcagtaaattgaacggag

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

30

5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

5

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

10

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.

20

Im nächsten Schritt wurde der Cnl1-B Promotor durch PCR mittels folgender Primer amplifiziert:

Cnl1-B 5': aggcctcaacgggtccggcggtatag

25

Cnl1-B 3': cccgggggtaacgctagcgggcccgatatcggatccatttttggtggtgattggtct

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

30

5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

5

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

10

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.

20

In einem weiteren Schritt wurde der OCS-Terminator für Cnl1B eingefügt. Dazu wurde die PCR mit folgenden Primer durchgeführt:

OCS2 5': aggcctcctgctttaatgagatatgcgagac

25 OCS2 3': cccgggaggacaatcagtaaattgaacggag

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

30 5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

5

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

10

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

15

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.

20

Im nächsten Schritt wurde der Cnl1-A Promotor durch PCR mittels folgender Primer amplifiziert:

Cnl1-B 5': aggcctcaacgggtccggcggtatagag

25

Cnl1-B 3': aggccttctagactgcaggcggccgcccgcatttttgggtggtgattgt

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

30

5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

5

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

10

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.

15

20

In einem weiteren Schritt wurde der OCS-Terminator für Cnl1A eingefügt. Dazu wurde die PCR mit folgenden Primer durchgeführt:

OCS2 5': ggcctcctgctttaatgagatatgcca

OCS2 3': aagcttggcgcgcccagctcgtcgcacggacaatcagtaaattgaacggaga

25

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

30

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

5 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
10 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
15 der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschlie-
ßend 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.

20 Das Plasmid *pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3* wurde im nächsten Schritt zur Klonie-
rung der $\Delta 6$ -, $\Delta 5$ -Desaturase und $\Delta 6$ -Elongase verwendet. Dazu wurde die $\Delta 6$ -
Desaturase aus *Phytium irregulare* (WO02/26946) mit folgenden PCR-Primern amplifi-
ziert:

25 D6Des(Pir) 5': agatctatggtggacctaagcctggagtg

D6Des(Pir) 3': ccatggcccgggttacatcgctgggaactcggtgat

Zusammensetzung des PCR-Ansatzes (50 μ l):

30 5,00 μ l Template cDNA

5,00 μ l 10x Puffer (Advantage-Polymerase) + 25mM $MgCl_2$

5,00 μ l 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

5

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

10

Das PCR-Produkt wurde zuerst für 2 h bei 37°C mit dem Restriktionsenzym BglIII 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 BglIII 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.

15

20

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:

25

D5Des(Tc) 5': gggatccatgggcaagggcagcgagggccg

D5Des(Tc) 3': ggcgccgacaccaagaagcaggactgagatc

Zusammensetzung des PCR-Ansatzes (50 µl):

30

5,00 µl Template cDNA

5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

5 Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

10 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 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

30 Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

5 0,50 µl Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C

10 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 NotI und
15 dann für 2 h bei 37°C mit dem Restriktionsenzym XbaI inkubiert. Der Vektor
pUC19-Cnl1_d6Des(Pir)_d5Des(Tc) wurde für 2 h bei 37°C mit dem Restriktionsenzym
NotI und für 2 h bei 37°C mit dem Restriktionsenzym XbaI inkubiert. Anschließend wur-
den das PCR-Produkt und der geschnittene Vektor durch Agarose-Gelelektrophorese
aufgetrennt und die entsprechenden DNA-Fragmente ausgeschnitten. Die Aufreinigung
20 der DNA erfolgte mittels Qiagen Gel Purification Kit gemäß Herstellerangaben. Anschlie-
ßend wurden Vektor und PCR-Produkt ligiert. Dazu wurde das Rapid Ligation Kit von
Roche verwendet. Das entstandene Plasmid pUC19-
Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurde durch Sequenzierung verifiziert.

25 Ausgehend von pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurde der binäre Vektor
für die Pflanzentransformation hergestellt. Dazu wurde
pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) für 2 h bei 37°C mit dem Restriktionsen-
zym AscI inkubiert. Der Vektor pGPTV wurde in gleicher Weise behandelt. Anschließend
wurden das Fragment aus pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) und der ge-
30 schnittene pGPTV-Vektor durch Agarose-Gelelektrophorese aufgetrennt und die ent-
sprechenden 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.

- 5 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

- 10 Cnl1_OCS 3': gtcgatcggacaatcagtaaattgaacggaga

Zusammensetzung des PCR-Ansatzes (50 µl):

5,00 µl Template cDNA

- 15 5,00 µl 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

5,00 µl 2mM dNTP

1,25 µl je Primer (10 pmol/µl)

0,50 µl Advantage-Polymerase (Clontech)

- 20 Reaktionsbedingungen der PCR:

Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

- 25 Anzahl der Zyklen: 35

- Das PCR-Produkt wurde für 2 h bei 37°C mit dem Restriktionsenzym Sall inkubiert. Der Vektor pUC19 wurde für 2 h bei 37°C mit dem Restriktionsenzym Sall 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
- 30

Ligation Kit von Roche verwendet. Das entstandene Plasmid pUC19-Cnl1_OCS wurde durch Sequenzierung verifiziert.

5 In einem weiteren Schritt wurde das $\Delta 12$ -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': ccatggtaaactctattacgatacc

10

Zusammensetzung des PCR-Ansatzes (50 μ l):

5,00 μ l Template cDNA

5,00 μ l 10x Puffer (Advantage-Polymerase) + 25mM MgCl₂

15

5,00 μ l 2mM dNTP

1,25 μ l je Primer (10 pmol/ μ l)

0,50 μ l Advantage-Polymerase (Clontech)

Reaktionsbedingungen der PCR:

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Anlagerungstemperatur: 1 min 55°C

Denaturierungstemperatur: 1 min 94°C

Elongationstemperatur: 2 min 72°C

Anzahl der Zyklen: 35

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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-

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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

- 5 pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) wurden für 2 h bei 37°C mit dem Restriktionsenzym Sall 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.
- 10 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

- 15 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 Ascl inkubiert. Der Vektor pGPTV wurde in gleicher Weise behandelt. Anschließend wurden das Fragment aus
- 20 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
- 25 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 Ascl 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 Ascl-Adapter (5'-ggcgcgcc; am 5'-Ende phosphoryliert, doppelsträngig) in den pENTR1A-Vektor ligiert. In diesen modifizierten wurde wie oben beschrieben schrittweise Gene in die Cnl-Kassette eingefügt und über Ascl 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.

- 5 Ein weiteres Konstrukt, das 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
10 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).
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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 Glas-
5 mühle, flüssigen Stickstoff und Mahlen oder über andere anwendbare Verfahren auf-
gebrochen 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 Lipidverbindun-
10 gen führt, die transmethylierte Lipide ergeben. Diese Fettsäuremethylester werden in
Petrolether extrahiert und schließlich einer GC-Analyse unter Verwendung einer Kapil-
larsäule (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 Ver-
15 wendung von Standards, die aus kommerziellen Quellen erhältlich sind (d.h. Sigma),
definiert werden.

Pflanzenmaterial wird zunächst mechanisch durch Mörsern homogenisiert, um es einer
20 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 Fettsäure-
methylester (FAME) werden in Petrolether extrahiert. Die extrahierten FAME werden
25 durch Gasflüssigkeitschromatographie mit einer Kapillarsäule (Chrompack, WCOT Fu-
sed 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 Fettsäuremethyles-
ter wird durch Vergleich mit entsprechenden FAME-Standards (Sigma) bestätigt. Die
Identität und die Position der Doppelbindung kann durch geeignete chemische Derivati-
30 sierung der FAME-Gemische z.B. zu 4,4-Dimethoxyoxazolin-Derivaten (Christie, 1998)
mittels GC-MS weiter analysiert werden.

Beispiel 9: Verwendung der optimierten $\Delta 5$ -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
5 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 Expressionskas-
sette 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) zusam-
10 mengesetzt. Dabei wurde der Promotor über die Restriktionsschnittstellen Sall/XbaI und
der Terminator über die Restriktionsschnittstellen BamHI/SmaI eingesetzt. An den Ter-
minator wurde außerdem ein Polylinker mit der XhoI Schnittstelle angehängt ('triple liga-
tion'). Das entstandene Plasmid pUC19-35S wurde dann für die Klonierung von PUFA
Genen eingesetzt. Es wurden parallel die offenen Leserahmen der $\Delta 6$ -Desaturase- (SEQ
ID NO. 1), der $\Delta 5$ -Desaturase- (SEQ ID NO. 51) und $\Delta 6$ -Elongase- (SEQ ID NO. 171)
15 Sequenzen über die EcoRV-Schnittstelle in pUC19-35S Vektoren eingefügt. Die ent-
standenen Plasmide pUC-D6, pUC-D5, pUC-E6(Tc) wurden für die Erstellung des binä-
ren Vektors pGPTV-35S_D6D5E6(Tc) verwendet. Dazu wurde der Vektor pGPTV mit
dem Enzym Sall, das Plasmid pUC-D6 mit Sall/XhoI verdaut und die korrekten Fragmen-
te ligiert. Das entstandene Plasmid pGPTV-D6 wurde anschließend mit Sall, das Plas-
20 mid pUC-D5 mit Sall/XhoI verdaut und die korrekten Fragmente ligiert. Das entstandene
Plasmid pGPTV-D6-D5 wurde dann ein weiteres Mal mit Sall verdaut, das Plasmid pUC-
E6(Tc) mit Sall/XhoI und die korrekten Fragmente ligiert. Aus diesen sequentiellen Klo-
nierungsschritten entstand der binäre Vektor pGPTV-D6D5E6(Tc), der für die Transfor-
mation eingesetzt wurde.

25

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.

30

In einer weiteren Ausführung wurde der offene Leserahmen der $\omega 3$ Des (SEQ ID NO.
193) in pUC19-35S kloniert. Das entstandene Plasmid pUC- $\omega 3$ Pi wurde über Sall/XhoI in

die binären Vektoren pGPTV-D6D5E6(Tc) und pGPTV-D6D5E6(Tp) übertragen. Die entstandenen Vektoren pGPTV-D6D5E6(Tc) ω 3Pi und pGPTV-D6D5E6(Tp) ω 3Pi wurden für die Pflanzentransformation eingesetzt.

- 5 In einer weiteren Ausführung wurde der offene Leserahmen der optimierten Δ 5-Elongase aus *Ostreococcus tauri* (Seq ID No. 64), sowie der offene Leserahmen der Δ 4-Desaturase aus *Thraustochytrium* sp. (Seq ID No. 77) in pUC19-35S kloniert. Die entstandenen Plasmide pUC-E5 und pUC-D4 wurden dann über Sall/XhoI entsprechend obiger Angaben in den Vektor pGPTV-D6D5E6(Tp) ω 3Pi übertragen. Der entstandene
- 10 Vektor pGPTV-D6D5E6(Tp) ω 3PiE5D4 wurde für die Pflanzentransformation eingesetzt.

Alle binären Vektoren wurden in *E. coli* DH5 α -Zellen (Invitrogen) nach Herstellerangaben transformiert. Positive Klone wurden durch PCR identifiziert und Plasmid-DNA isoliert (Qiagen Dneasy).

- 15 **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).

20

Zur Erzeugung transgener Pflanzen wurde der binäre Vektor pGPTV-D6D5E6(Tp) ω 3PiE5D4 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 Übernachtskultur einer positiv

25 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 cm²) 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

30 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 μ M 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.

5

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.

10

b) Erzeugung transgener *Orychopragmus violaceus* Pflanzen

15

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-

20

D6D5E6(Tp) ω 3PiE5D4 in *Agrobacterium tumefaciens* C58C1:pGV2260 transformiert (Deblaere et al. (1984) Nucl. Acids. Res. 13: 4777-4788). Zur Transformation von *Orychopragmus violaceus* wurde eine 1:50 Verdünnung einer Übernachtskultur einer positiv transformierten Agrobakterienkolonie in Murashige-Skoog Medium (Murashige und Skoog (1962) Physiol. Plant. 15: 473) mit 3 % Saccharose (3MS-Medium) verwendet.

25

Petiolen oder Hypokotyledonen frisch gekeimter steriler Pflanzen (zu je ca. 1 cm²) 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

30

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 μ M 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) ω 3PiE5D4 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 *Orychopragmus*- bzw. *Arabidopsis*-Pflanzen

Die Extraktion von Blattmaterial transgener *Orychopragmus violaceus* und *Arabidopsis thaliana* Pflanzen transformiert mit pGPTV-D6D5E6(Tp) ω 3PiE5D4 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 $\Delta 5$ -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 transformiert 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 mehrfach ungesättigten Fettsäuren (PUFA) gezeigt werden. Überraschenderweise resultierte die Verwendung der modifizierten Elongase-Sequenz OtE-LO2.2 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.-% mehrfach ungesättigte 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 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% CaCl₂ (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 analysiert 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: $sn-1 + sn-3 = (TAG \times 3 - MAG) / 2$.

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)
5 und Phosphatidylcholin (PC) wurden unter N₂ getrocknet und in 0.5 ml Boratpuffer
(0.5M, pH 7.5, enthaltend 0.4 mM CaCl₂) resuspendiert. Nach einer kurzen Ultraschall-
behandlung wurden 5U der Phospholipase A2 aus dem Gift von Naja mossambica (Sig-
ma 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
10 HCl abgebrochen, und das Reaktionsgemisch wurde mit Chloroform:Methanol (2:1 v/v)
extrahiert. Die verdauten Phospholipide wurden durch TLC in Chloro-
form:Methanol:Ammoniak:Wasser (70:30:4:2 v/v/v/v) getrennt und Punkte, die den frei-
gesetzten freien Fettsäuren und Lysophospholipiden entsprachen, wurden durch Kratzen
entfernt und direkt transmethyliert.

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Die Positionsanalyse der Phospholipide zeigte eine Akkumulation von EPA und DHA in
der sn-2 Position von Phosphatidylcholin (PC), während DHA in Phosphatidyl-
ethanolamin (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
20 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

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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
30 (DGDG) wurden unter Stickstoff getrocknet und in 0.5 ml Diethylether gelöst. Dann wur-
den 25 Einheiten der Lipase aus Rhizopus arrhizus (Sigma 62305), resuspendiert in 2 ml
Boratepuffer (50 mM, pH 7.5 enthaltend 2 mM CaCl₂), 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 Digalaktodiacylglycerolen (DGDG) zu finden und konnte nicht in den Monogalaktodiacylglycerolen (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 <i>Ostreococcus tauri</i> 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 <i>Orychophragmus violaceus</i>											
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 <i>Arabidopsis thaliana</i>											
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 Triacylglyceride aus Blattmaterial von transgenen *O. violaceus* Pflanzen.

TAG	16:0	18:0	18:1n-3	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	2,35	9,51									
sn-2	1,62	0,64	8,33	1,61	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	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	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	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	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	27,57	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.

MEDG	160	161	162	163	180	181n9	181n7	182n9	182n6	183n6	183n3	184n3	203n6	204n6	204n3	205n3
wt	264	0,13	1,23	30,72	0,33	0,35	0,26	3,81	60,52							
sn1	0,00	0,05	0,00	7,11	0,35	0,31	0,41	4,60	87,30							
sn2	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	1,64	44,82	1,88	2,73	0,04	0,30	0,50	5,03	
sn1	1,22	0,29	0,54	4,79	1,51	1,15	0,93	2,80	80,19	0,14	0,08	0,08	0,17	0,87	3,86	
sn2	7,11	0,11	1,61	62,82	0,34	0,31	0,11	0,47	9,46	3,62	5,48	0,00	0,43	0,14	6,31	

DEEG	160	161	162	163	180	181n9	181n7	182n9	182n6	183n6	183n3	184n3	203n6	204n6	204n3	205n3
wt	17,67	0,19	0,38	2,15	1,61	0,51	0,94	5,56	70,71							
sn1	16,84	0,25	0,50	2,52	2,21	0,55	1,75	6,07	68,74	0,00						
sn2	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	6,28	54,66	3,55	0,00	0,00	0,00	0,00	2,18	5,36
sn1	22,74	0,17	0,23	0,48	4,55	1,71	2,32	9,22	56,06	0,23	0,27	0,00	0,00	0,36	1,23	
sn2	14,27	0,00	0,00	4,77	1,12	1,00	0,46	3,33	53,26	6,88	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	γ 18:3	α 18:3	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

5

	Lipid Profile(%)								
	20:3 (0,11,14)	20:3 (11,14,17)	20:4 (ARA4) (5,8,11,14)	20:4 (ETE4) (0,11,14,17)	20:5 (EPA) (5,8,11,14,17)	22:1	22:4	22:5	22:6
1,1	0,5	3,1	4,8	0,6	4,8	0,0	1,5	2,0	1,6
2,0	0,9	4,2	4,1	1,0	4,1	0,0	3,1	3,5	1,9
1,3	0,7	4,1	4,5	0,5	4,5	0,0	2,7	2,8	1,6
1,4	0,6	3,6	4,4	0,6	4,4	0,0	2,4	2,5	1,6

Patentansprüche

1. Verfahren zur Herstellung von Eicosapentaensäure, Docosapentaensäure und/oder Docosahexaensäure in einer transgenen Pflanze, umfassend das Bereitstellen in
5 der Pflanze von mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 6$ -Desaturase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert; mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Desaturase-Aktivität kodiert; und mindestens einer Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Elongase-Aktivität und optional für eine $\Delta 4$ -Desaturase kodiert,
10 wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -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.
15
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
20 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.
- 25 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-, Peroxiredoxin-, Legumin-, Fad3-, Conlinin- oder Oleosin-Promotor handelt.
30

7. Verfahren nach Anspruch 6, wobei der Gehalt an mehrfach ungesättigten C22-Fettsäuren im Samenöl 5 Gew.-% oder mehr des Samenölgehalts beträgt.
- 5 8. Verfahren nach einem der vorangehenden Ansprüche, wobei zusätzlich eine oder mehrere Nukleinsäuresequenzen kodierend für ein Polypeptid mit der Aktivität einer ω 3-Desaturase und/oder einer Δ 4-Desaturase in der Pflanze bereitgestellt werden.
9. Verfahren nach Anspruch 8, wobei der Gehalt an Docosahexaensäure im Samenöl 1
10 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.
- 15 11. Verfahren nach einem der vorangehenden Ansprüche, wobei die Pflanze eine Ölproduzierende Pflanze ausgewählt aus der Gruppe bestehend aus *Brassica napus*, *Brassica juncea* und *Glycine max* ist.
- 20 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.
- 25 .
13. Isoliertes Nukleinsäuremolekül umfassend eine Nukleinsäuresequenz gemäß Seq ID No. 64.
14. Rekombinantes Nukleinsäuremolekül, umfassend:

103

- a) eine oder mehrere Kopien von mindestens einem in Pflanzenzellen aktiven Promotor,
- b) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -Desaturase-Aktivität kodiert,
- 5 c) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 5$ -Desaturase-Aktivität kodiert,
- d) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert,
- e) mindestens eine Nukleinsäuresequenz, die für ein Polypeptid mit einer $\Delta 5$ -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
- 10 e) eine oder mehrere Kopien von mindestens einer Terminatorsequenz.
- 15 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 $\omega 3$ -Desaturase und/oder einer $\Delta 4$ -Desaturase.
- 20 17. Transgene Pflanze enthaltend ein rekombinantes Nukleinsäuremolekül nach einem der Ansprüche 22 bis 25 oder enthaltend die in Seq ID No. 64 angegebene Nukleinsäuresequenz.
- 25 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.
- 30

Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren

ZUSAMMENFASSUNG

5 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 $\Delta 6$ -Desaturase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 6$ -Elongase-Aktivität kodiert; mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Desaturase-Aktivität kodiert; und mindestens eine Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -Elongase-Aktivität kodiert, wobei die Nukleinsäuresequenz, welche für ein Polypeptid mit einer $\Delta 5$ -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 $\Delta 4$ -Desaturase-Aktivität kodiert, in die Pflanze eingebracht.

20

PF58307.txt
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 <120> Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren
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 <160> 199
 <170> Bi omax Patent Tool according to Patent IN 3.1 format
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 Asn Leu Ile Gly Cys Leu Val Gly Asn Ala Trp Gl n Gly Phe Ser Met
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Seite 1

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Seite 7

PF58307. txt

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 <213> Cunnin ghamel l a echi nul at a

<220>
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 <222> (1) . . (1401)

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 Leu Lys Ala Tyr Gln Ala Gly Asp Lys Asn Ala Asp Lys Phe Leu Ile 20
 20 gt c gac aac aag gt g t ac gac at c acg gat t t c att gct gat cat cct 144
 Val Asp Asn Lys Val Tyr Asp Ile Thr Asp Phe Ile Ala Asp His Pro 35
 35 ggc ggt gct cag gt c at c t ct acg cac att ggc aag gat gcc t ct gat 192
 Gly Gly Ala Gln Val Ile Ser Thr His Ile Gly Lys Asp Ala Ser Asp 50
 50 gt g ttt cat gcg at g cat ccc gag t ct gcg t ac gaa t t g ct t gcg aat 240
 Val Phe His Ala Met His Pro Glu Ser Ala Tyr Glu Leu Leu Ala Asn 65
 65 t gc t ac gt t ggc gat ct t gct gcg gac cat gct ggt gt g cag ggc gaa 288
 Cys Tyr Val Gly Asp Leu Ala Ala Asp His Ala Gly Val Gln Gly Glu 85
 85 t t g gt g aat ggt gt a cac aaa aag t cc aag gca t t t gcg gac gag at g 336
 Leu Val Asn Gly Val His Lys Lys Ser Lys Ala Phe Ala Asp Glu Met 100
 100 cga t cg t t g cgc gag cgt ct t gag acg gag ggt gcc t t t aat ggc agt 384
 Arg Ser Leu Arg Glu Arg Leu Glu Thr Glu Gly Ala Phe Asn Gly Ser 115
 115 gt t ccg ttt t ac att t ac aag gt g gt g t cg acg t t g gcc att ggt gcg 432
 Val Pro Phe Tyr Ile Tyr Lys Val Val Ser Thr Leu Ala Ile Gly Ala 130
 130 acg ggt ct g gcc at g ct g t ac t at ggc ggc cat t cg acg t cg gt c gt g 480
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PF58307.txt

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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 Gn Gn Cys Gly Trp
165
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 Gn Ala Phe Ala Asp His Thr Val
180
aat gac gtg atg att gcg ttt ttg ggt gga ttc tgc caa ggt ttc tgc      624
Asn Asp Val Met Ile Ala Phe Leu Gly Gly Phe Cys Gn Gly Phe Ser
195
ctg tgc 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
gtc cac ggc cac gat ccc gat atc gat acg gcc cct gtc ct c ct c tgg      720
Val His Gly His Asp Pro Asp Ile Asp Thr Ala Pro Val Leu Leu Trp
225
gac gaa ttt gcc acc gcc aat ttc tat gga aac ct c gag ggt caa aag      768
Asp Gu Phe Ala Thr Ala Asn Phe Tyr Gly Asn Leu Gu Gly Gn Lys
245
gat tgc gcg ttc tgc cgc ttc att gcc gaa cac gtg ct c ccc tac cag      816
Asp Ser Ala Phe Ser Arg Phe Ile Ala Gu His Val Leu Pro Tyr Gn
260
acg cgc tat tac ttt ttt gtc ct c ggc ttt gcg cgt ct c tgc tgg gcc      864
Thr Arg Tyr Tyr Phe Phe Val Leu Gly Phe Ala Arg Leu Ser Trp Ala
275
atc caa tgc ct c caa tac tgc ttc act gtc ggc aca ct c aac aag tca      912
Ile Gn Ser Leu Gn Tyr Ser Phe Thr Val Gly Thr Leu Asn Lys Ser
290
aag acc ct c aat ctg ttt gag cgc acc atg ctt gtc agc cat tgg atc      960
Lys Thr Leu Asn Leu Phe Gu Arg Thr Met Leu Val Ser His Trp Ile
305
ttg ttc acc act tgg acg ct c ct c 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
gtc atg ttc ttt gtc gtc agc cag gcc acc acc ggc tac gcg ct t gcc      1056
Val Met Phe Phe Val Val Ser Gn Ala Thr Thr Gly Tyr Ala Leu Ala
340
ctc gtt ttc gcc atg aac cac tgc ggc atg ccc gtc ct c acc caa gaa      1104
Leu Val Phe Ala Met Asn His Ser Gly Met Pro Val Leu Thr Gn Gu
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cag gcc caa aag atg gag ttc tac gag atc cag gtc gt c acc gcc cgc      1152
Gn Ala Gn Lys Met Gu Phe Tyr Gu Ile Gn Val Val Thr Ala Arg
370
gac gtc acc ct c ggt gcc ctt ggc gac tgg ttc tgc ggc ggc ct c aac      1200
Asp Val Thr Leu Gly Ala Leu Gly Asp Trp Phe Cys Gly Gly Leu Asn
385
tat caa atc gag cac cat gta ttc cct gat atg ccc cgc cac tac ctg      1248
Tyr Gn Ile Gu His His Val Phe Pro Asp Met Pro Arg His Tyr Leu
405
ccc aag gtc aag cct caa gtc aag gct ct c tgc aaa aaa cac aac att      1296
Pro Lys Val Lys Pro Gn Val Lys Ala Leu Cys Lys Lys His Asn Ile
420
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 Gu Val Leu Gn
435
acc ttg gat gtt gtc caa aag ct c tgc gca agt tct ct c aaa aga tgt      1392
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ttt aga tga
Phe Arg
465

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<213> Cunnin ghamel I a echi nul at a

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PF58307. txt

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Gly Gly Ala Gln Val Ile Ser Thr His Ile Gly Lys Asp Ala Ser Asp
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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 Gln 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 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
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Phe Arg
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<220>

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<222> (1)..(1398)

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Lys	Thr	Leu	Pro	Lys	Phe	Thr	Leu	Glu	Glu	Ile	Gln	Lys	His	Arg	Thr	
			20					25					30			
ccg	cag	gat	gcg	tgg	at g	gt g	cac	cac	aac	aag	gt g	t ac	gat	gt g	t cc	144
Pro	Gln	Asp	Ala	Trp	Met	Val	His	His	Asn	Lys	Val	Tyr	Asp	Val	Ser	
			35				40					45				
aac	t at	at g	gac	cac	ccc	ggc	ggt	ct g	gt g	at c	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	at g	acc	gac	gt c	ttc	gcg	gcg	ttc	cac	ccg	ccc	t cg	gcg	ttc	240
Asp	Asp	Met	Thr	Asp	Val	Phe	Ala	Ala	Phe	His	Pro	Pro	Ser	Ala	Phe	
					70					75					80	
aac	ttc	at g	gac	aag	ttc	ttg	att	ggc	gt g	gt g	gac	agc	aag	ggc	t cg	288
Asn	Phe	Met	Asp	Lys	Phe	Leu	Ile	Gly	Val	Val	Asp	Ser	Lys	Gly	Ser	
				85				90						95		
t cg	ccg	cag	ct c	cag	aag	gac	gcg	t cc	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			
t ac	cgc	aat	ct g	cga	gt c	cag	ct c	aag	aag	gcc	ggc	at g	ttc	aag	gcc	384
Tyr	Arg	Asn	Leu	Arg	Val	Gln	Leu	Lys	Lys	Ala	Gly	Met	Phe	Lys	Ala	
			115				120					125				
t cg	agc	ct c	ttc	t ac	acc	t ac	aag	gt g	ct c	t cg	acc	ct g	gcg	ct g	t gc	432
Ser	Ser	Leu	Phe	Tyr	Thr	Tyr	Lys	Val	Leu	Ser	Thr	Leu	Ala	Leu	Cys	
			130			135				140						
ct c	gt g	t cc	t gg	ggc	ct c	gt g	ct g	ggg	t cg	gac	cac	ttc	ggt	gt g	cac	480
Leu	Val	Ser	Trp	Gly	Leu	Val	Leu	Gly	Ser	Asp	His	Phe	Gly	Val	His	
				145	150			155		160					160	
ct c	gt g	ggc	gct	ct c	ttc	ct c	gcg	ct c	ttc	t gg	cag	cag	t gc	ggc	t gg	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	ct g	cac	cac	cag	gt c	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	ct c	gcc	ggt	at c	at g	at c	ggc	aac	gt g	t gg	cag	ggc	ttc	t cc	624
Gly	Asp	Leu	Ala	Gly	Ile	Met	Ile	Gly	Asn	Val	Trp	Gln	Gly	Phe	Ser	
			195			200						205				
gt g	gcc	t gg	t gg	aag	aac	aag	cac	aac	acc	cac	cac	t cg	gt g	ccg	aac	672
Val	Ala	Trp	Trp	Lys	Asn	Lys	His	Asn	Thr	His	His	Ser	Val	Pro	Asn	
			210			215						220				
ct c	t ac	gag	t cg	cag	ccc	gac	gcc	gcc	gac	ggc	gat	ccg	gac	at c	gac	720
Leu	Tyr	Glu	Ser	Gln	Pro	Asp	Ala	Ala	Asp	Gly	Asp	Pro	Asp	Ile	Asp	
				225	230				235						240	
acc	at g	ccc	ct c	ct c	gcc	t gg	t cg	ct g	cgc	at g	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	ct a	t cc	cgg	t gg	ttc	gt g	t cc	cac	cag	gcc	ttc	t gc	t ac	ttc	816
Asn	Ala	Leu	Ser	Arg	Trp	Phe	Val	Ser	His	Gln	Ala	Phe	Cys	Tyr	Phe	
			260				265						270			
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Pro	Ile	Leu	Gly	Leu	Ala	Arg	Leu	Ser	Trp	Leu	Glu	Gly	Ser	Phe	Ser	
			275				280					285				
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Phe	Val	Phe	Ser	Asn	Pro	Leu	Ala	Trp	Lys	Thr	Lys	Asn	Leu	Asp	Val	
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gcc	aag	cag	ct c	gt g	acc	aac	ccc	ct g	ttg	gag	cag	gcc	ggc	ct c	ct g	960
Ala	Lys	Gln	Leu	Val	Thr	Asn	Pro	Leu	Leu	Glu	Gln	Ala	Gly	Leu	Leu	
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gt c	cac	t ac	gcc	t gg	gt c	ttc	gcc	ct c	t gc	gcg	t gc	acg	ggc	t cc	ct g	1008
Val	His	Tyr	Ala	Trp	Val	Phe	Ala	Leu	Cys	Ala	Cys	Thr	Gly	Ser	Leu	
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gt g	cgc	gcc	ct g	gcg	ttc	ttc	ttc	gt g	gcc	acc	t gc	acc	agc	ggc	ct t	1056

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Val	Arg	Ala	Leu	Ala	Phe	Phe	Phe	Val	Ala	Thr	Cys	Thr	Ser	Gly	Leu		
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		
		340					360					365					
gag	gcc	aat	gcc	cgg	ccg	gac	ttc	tgg	aag	ctc	cag	gtg	acc	acc	acg		1152
Gu	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		400
					390					395							
cag	ttc	cag	gtg	gag	cac	cac	ctc	ttc	ccc	tcg	ctg	ccc	cgg	cac	aac		1248
Gln	Phe	Gln	Val	Gu	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	Gu	Ile	Val	Thr	Ala	Phe	Cys	Lys	Gu	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	Gu	Ala	Asp	Leu	Leu	Thr	Gly	Thr	Lys	Gu	Ile	Leu		
		435					440					445					
tgc	tgc	ttg	tgc	gag	gta	acg	acg	gag	ttc	ctc	gac	gag	ttc	cca	gcc		1392
Ser	Cys	Leu	Ser	Gu	Val	Thr	Thr	Gu	Phe	Leu	Asp	Gu	Phe	Pro	Ala		
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Met																	
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 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 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 Gu 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 Gu 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
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 260 265 270
 Pro Ile Leu Gly Leu Ala Arg Leu Ser Trp Leu Gu Gly Ser Phe Ser

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 Ala Lys Gln Leu Val Thr Asn Pro Leu Leu Glu Gln Ala Gly Leu Leu
 305 310 315
 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
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<213> Thal assi osi ra pseudonana

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<222> (1)..(1455)

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 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 at t 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
 t cg agt aag atg tat tat gca tac aag tgc t cg 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 t cg 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 t cg gct ct c ttg ttg gga ttg ttc t gg cag cag t gt gga 528
 His Ile Gly Ser Ala Leu Leu Leu Gly Leu Phe Trp Gln Gln Cys Gly

PF58307. txt

				165					170					175					
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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	Ala	Val	Pro					
	210					215					220								
aac	ttg	cac	aac	tct	tcc	ttg	gac	agt	cag	gat	ggg	gat	ccc	gat	att				720
Asn	Leu	His	Asn	Ser	Ser	Leu	Asp	Ser	Gln	Asp	Gly	Asp	Pro	Asp	Ile				240
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							
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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				320
305					310					315									
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				
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Phe	Phe	Thr	Ala	Thr	Cys	Ser	Ser	Gly	Leu	Phe	Leu	Ala	Leu	Val	Phe				
		355					360					365							
gga	ttg	gga	cac	aac	ggg	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	ggg	gga				1200
Asp	Phe	Trp	Gln	Leu	Gln	Val	Thr	Thr	Thr	Arg	Asn	Ile	Ile	Gly	Gly				400
385					390					395					400				
cac	ggc	att	ccc	caa	ttc	ttt	gtg	gat	tgg	ttc	tgc	ggg	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	ggg	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	ggg	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				480
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 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
 Gn Gly Ser Gn Ala Met Met Lys Lys Phe Tyr Ile Gly Asp Leu Ile
 85 90 95
 Pro Gu Ser Val Gu His Lys Asp Gn Arg Gn Leu Asp Phe Gu 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 Gn Gn Cys Gly
 165 170 175
 Trp Leu Ala His Asp Phe Leu His His Gn Val Phe Lys Gn Arg Lys
 180 185 190
 Tyr Gly Asp Leu Val Gly Ile Phe Trp Gly Asp Leu Met Gn Gly Phe
 195 200 205
 Ser Met Gn Trp Trp Lys Asn Lys His Asn Gly His Ala Val Pro
 210 215 220
 Asn Leu His Asn Ser Ser Leu Asp Ser Gn Asp Gly Asp Pro Asp Ile
 225 230 235 240
 Asp Thr Met Pro Leu Leu Ala Trp Ser Leu Lys Gn Ala Gn Ser Phe
 245 250 255
 Arg Gu Ile Asn Lys Gly Lys Asp Ser Thr Phe Val Lys Tyr Ala Ile
 260 265 270
 Lys Phe Gn Ala Phe Thr Tyr Phe Pro Ile Leu Leu Leu Ala Arg Ile
 275 280 285
 Ser Trp Leu Asn Gu Ser Phe Lys Thr Ala Phe Gly Leu Gly Ala Ala
 290 295 300
 Ser Gu Asn Ala Lys Leu Gu Leu Gu Lys Arg Gly Leu Gn Tyr Pro
 305 310 315 320
 Leu Leu Gu 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 Gn Leu Gn Val Thr Thr Thr Arg Asn Ile Ile Gly Gly
 385 390 395 400
 His Gly Ile Pro Gn Phe Val Asp Trp Phe Cys Gly Gly Leu Gn
 405 410 415
 Tyr Gn Val Asp His His Leu Phe Pro Met Met Pro Arg Asn Asn Ile
 420 425 430
 Ala Lys Cys His Lys Leu Val Gu Ser Phe Cys Lys Gu Trp Gly Val
 435 440 445
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 Gu Gl n Gl n Gl n Gl n Gl n Gl n Gl n Ser Ser Pro Gu Ala Ser Thr Pro
 35 40 45
 gcg gcg cag cag gag aaa t cc at c agt aga gaa t cc at c ccc gag ggc 192
 Ala Ala Gl n Gl n Gu Lys Ser Ile Ser Arg Gu Ser Ile Pro Gu Gly
 50 55 60
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 Phe Leu Thr Val Gu Gu Val Ser Lys His Asp Asn Pro Ser Asp Cys
 65 70 75 80
 t gg at c gt c at c aac gac aag gt g t ac gac gt g agc gca t t c ggg aag 288
 Tr p Ile Val Ile Asn Asp Lys Val Tyr Asp Val Ser Ala Phe Gly Lys
 85 90 95
 acg cat ccg ggc ggc cct gt g at c t t c acg cag gcc ggc cgc gac gcc 336
 Thr His Pro Gly Gly Pro Val Ile Phe Thr Gl n Ala Gly Arg Asp Ala
 100 105 110
 acg gat t ct t t c aag gt t t t c cac t cc gcc aag gcg t gg cag t t t ct c 384
 Thr Asp Ser Phe Lys Val Phe His Ser Ala Lys Ala Tr p Gl n Phe Leu
 115 120 125
 cag gac ct g t ac at c gga gat ct g t ac aat gcc gag cca gt g t cg gag 432
 Gl n Asp Leu Tyr Ile Gly Asp Leu Tyr Asn Ala Gu Pro Val Ser Gu
 130 135 140
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 145 150 155 160
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 Phe Ala Ile Leu Ala Ala Ser Leu Ala Val Ile Ala Tr p Ser Gl n Thr
 180 185 190
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 Tyr Leu Ala Val Leu Cys Ser Ser Phe Leu Leu Ala Leu Phe Tr p Gl n
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 Gl n Cys Gly Tr p Leu Ser His Asp Phe Leu His His Gl n Val Thr Gu
 210 215 220
 aac cga t cg ct c aac acg t ac t t c ggc ggc ct g t t c t gg ggt aac t t c 720
 Asn Arg Ser Leu Asn Thr Tyr Phe Gly Gly Leu Phe Tr p Gly Asn Phe
 225 230 235 240
 gcc cag ggc t ac agc gt g gga t gg t gg aag acc aag cac aat gt g cac 768
 Ala Gl n Gly Tyr Ser Val Gly Tr p Tr p Lys Thr Lys His Asn Val His
 245 250 255
 cac gcg gcc acg aac gaa t gc gac gac aag t at cag ccc at c gat ccc 816
 His Ala Ala Thr Asn Gu Cys Asp Asp Lys Tyr Gl n Pro Ile Asp Pro
 260 265 270
 gac at c gac acc gt g ccc ct g ct c gcc t gg agc aag gaa at c t t g gcc 864
 Asp Ile Asp Thr Val Pro Leu Leu Ala Tr p Ser Lys Gu Ile Leu Ala
 275 280 285
 acc gt c gac gac caa t t c t t c cga t cg at c at c agc gt g cag cac ct t 912
 Thr Val Asp Asp Gl n Phe Phe Arg Ser Ile Ile Ser Val Gl n His Leu
 290 295 300
 ct g t t c t t c ccg ct c ct c t t c t t g gca aga t t c agc t gg ct g cat t cg 960
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 305 310 315 320
 agt t gg gcc cac gcc agc aac t t c gag at g cct cgg t ac at g aga t gg 1008
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 325 330 335

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 340 345 350

gcc ttc tac att ttg ccc at c ccc cag gcc at c tgc tgg ct c ttc ttg 1104
 Al a Phe Tyr lle Leu Pro lle Pro Gn Al a lle Cys Trp Leu Phe Leu
 355 360 365

t cg caa ctg ttt tgc ggc gct ctg ct c agc att gt c ttc gt g at c agc 1152
 Ser Gn Leu Phe Cys Gy Al a Leu Leu Ser lle Val Phe Val lle Ser
 370 375 380

cac aat ggc at g gat gt g t ac aac gac ccc cgg gac ttc gt g acg gcc 1200
 Hi s Asn Gy Met Asp Val Tyr Asn Asp Pro Arg Asp Phe Val Thr Al a
 385 390 395 400

caa gt c acc t cg acc aga aac at c gaa ggc aac ttc ttc aac gac tgg 1248
 Gn Val Thr Ser Thr Arg Asn lle Gu Gy Asn Phe Phe Asn Asp Trp
 405 410 415

ttc acc gga ggc ctg aac agg cag at t gag cac cat ct g ttt ccg tct 1296
 Phe Thr Gy Gy Leu Asn Arg Gn lle Gu Hi s Hi s Leu Phe Pro Ser
 420 425 430

ct t ccg agg cac aac ct c gcc aag gt c gcg cca cac gt c aag gcg ct c 1344
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 435 440 445

tgc gcc aag cac ggt ttg cat t ac gaa gaa ttg agt ct g ggc acg gga 1392
 Cys Al a Lys Hi s Gy Leu Hi s Tyr Gu Gu Leu Ser Leu Gy Thr Gy
 450 455 460

gt c tgt cgt gt c ttc aat cgg ct a gt a gag gt a gca t ac gct gcg aaa 1440
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 Val

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 35 40 45
 Al a Al a Gn Gn Gu Lys Ser lle Ser Arg Gu Ser lle Pro Gu Gy
 50 55 60
 Phe Leu Thr Val Gu Gu Val Ser Lys Hi s Asp Asn Pro Ser Asp Cys
 65 70 75 80
 Trp lle Val lle Asn Asp Lys Val Tyr Asp Val Ser Al a Phe Gy Lys
 85 90 95
 Thr Hi s Pro Gy Gy Pro Val lle Phe Thr Gn Al a Gy Arg Asp Al a
 100 105 110
 Thr Asp Ser Phe Lys Val Phe Hi s Ser Al a Lys Al a Trp Gn Phe Leu
 115 120 125
 Gn Asp Leu Tyr lle Gy Asp Leu Tyr Asn Al a Gu Pro Val Ser Gu
 130 135 140
 Leu Val Lys Asp Tyr Arg Asp Leu Arg Thr Al a Phe Met Arg Ser Gn
 145 150 155 160
 Leu Phe Lys Ser Ser Lys Met Tyr Tyr Val Thr Lys Cys Val Thr Asn
 165 170 175
 Phe Al a lle Leu Al a Al a Ser Leu Al a Val lle Al a Trp Ser Gn Thr
 180 185 190
 Tyr Leu Al a Val Leu Cys Ser Ser Phe Leu Leu Al a Leu Phe Trp Gn
 195 200 205
 Gn Cys Gy Trp Leu Ser Hi s Asp Phe Leu Hi s Hi s Gn Val Thr Gu
 210 215 220
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 225 230 235 240
 Al a Gn Gy Tyr Ser Val Gy Trp Trp Lys Thr Lys Hi s Asn Val Hi s

PF58307. txt

245 250 255
 H i s A l a A l a T h r A s n G u C y s A s p A s p L y s T y r G l n P r o I l e A s p P r o
 260 265 270
 A s p I l e A s p T h r V a l P r o L e u L e u A l a T r p S e r L y s G u I l e L e u A l a
 275 280 285
 T h r V a l A s p A s p G l n P h e P h e A r g S e r I l e I l e S e r V a l G l n H i s L e u
 290 295 300
 L e u P h e P h e P r o L e u L e u P h e L e u A l a A r g P h e S e r T r p L e u H i s S e r
 305 310 315
 S e r T r p A l a H i s A l a S e r A s n P h e G u M e t P r o A r g T y r M e t A r g T r p
 320 325 330 335
 A l a G u L y s A l a S e r L e u L e u G y H i s T y r G y A l a S e r I l e G y A l a
 340 345 350
 A l a P h e T y r I l e L e u P r o I l e P r o G l n A l a I l e C y s T r p L e u P h e L e u
 355 360 365
 S e r G l n L e u P h e C y s G y A l a L e u L e u S e r I l e V a l P h e V a l I l e S e r
 370 375 380
 H i s A s n G y M e t A s p V a l T y r A s n A s p P r o A r g A s p P h e V a l T h r A l a
 385 390 395 400
 G l n V a l T h r S e r T h r A r g A s n I l e G u G y A s n P h e P h e A s n A s p T r p
 405 410 415
 P h e T h r G y G y L e u A s n A r g G l n I l e G u H i s H i s L e u P h e P r o S e r
 420 425 430
 L e u P r o A r g H i s A s n L e u A l a L y s V a l A l a P r o H i s V a l L y s A l a L e u
 435 440 445
 C y s A l a L y s H i s G y L e u H i s T y r G u G u L e u S e r L e u G y T h r G y
 450 455 460
 V a l C y s A r g V a l P h e A s n A r g L e u V a l G u V a l A l a T y r A l a A l a L y s
 465 470 475 480
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 1 5 10 15
 c t g a t t a a t c a a a a a c a t c g a g a t g g a g a t a a a a g t g c g a t g a a g t t c 96
 L e u I l e A s n G l n L y s H i s A r g A s p G y A s p L y s S e r A l a M e t L y s P h e
 20 25 30
 a t t a t c a t t g a t c g t a a g g t g t a c g a t g t g a c t g a a t t c t t g g a a g a t 144
 I l e I l e I l e A s p A r g L y s V a l T y r A s p V a l T h r G u P h e L e u G u A s p
 35 40 45
 c a t c c t g g t g g t g c a c a a g t a t t a c t g a c a c a c g t t g g a a a a g a t g c a 192
 H i s P r o G y G y A l a G l n V a l L e u L e u T h r H i s V a l G y L y s A s p A l a
 50 55 60
 t c t g a t g t a t t t c a t g c c a t g c a t c c c g a g t c a g c a t a t g a a a t c t t g 240
 S e r A s p V a l P h e H i s A l a M e t H i s P r o G u S e r A l a T y r G u I l e L e u
 65 70 75 80
 a a c a a t t a t t t t g t a g g a g a t g t a a a a g a t g c a c a t g t a a a g a g a c c 288
 A s n A s n T y r P h e V a l G y A s p V a l L y s A s p A l a H i s V a l L y s G u T h r
 85 90 95
 c c t t c t g c t c a a t t t g c t t c a g a a a t g c g t c a a c t t c g g g a t c a a t t g 336
 P r o S e r A l a G l n P h e A l a S e r G u M e t A r g G l n L e u A r g A s p G l n L e u
 100 105 110
 a a a a a a g a a g g t t a t t t t c a t t c t a g t a a a g c t t a t t a t t a c a a g 384
 L y s L y s G u G y T y r P h e H i s S e r S e r L y s A l a T y r T y r T y r V a l T y r L y s
 115 120 125
 g t c c t c t c t a c t c t t g c t c t t t g t g c a g c t g g t c t t a c t c t t t t g t a t 432
 V a l L e u S e r T h r L e u A l a L e u C y s A l a A l a G y L e u T h r L e u L e u T y r

PF58307. txt

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145					150					155					160	
g t t	g g t	at c	t t t	t g g	caa	caa	t g t	g g t	t g g	t t g	gct	cat	gat	t t t	g g a	528
Val	Gl y	I l e	Phe	Tr p	Gl n	Gl n	Cys	Gl y	Tr p	Leu	Al a	Hi s	Asp	Phe	Gl y	
				165					170					175		
cat	cat	caa	t gc	t t t	gaa	gat	cgc	t ct	t gg	aat	gat	g t t	ct t	g t t	g t t	576
Hi s	Hi s	Gl n	Oys	Phe	Gl u	Asp	Arg	Ser	Tr p	Asn	Asp	Val	Leu	Val	Val	
			180					185					190			
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Phe	Leu	Gl y	Asn	Phe	Cys	Gl n	Gl y	Phe	Ser	Leu	Ser	Tr p	Tr p	Lys	Asn	
		195					200					205				
aaa	cat	aac	act	cac	cac	gct	agt	acg	aat	g t t	cat	gga	cat	gat	ccc	672
Lys	Hi s	Asn	Thr	Hi s	Hi s	Al a	Ser	Thr	Asn	Val	Hi s	Gl y	Hi s	Asp	Pro	
	210					215					220					
gat	at t	gac	act	gct	cct	gt c	t t a	t t a	t g g	gat	gaa	t at	gca	t ct	gca	720
Asp	I l e	Asp	Thr	Al a	Pro	Val	Leu	Leu	Tr p	Asp	Gl u	Tyr	Al a	Ser	Al a	
225					230					235					240	
gct	t at	t at	gcc	t ca	ct g	gat	gaa	gaa	ccc	aca	at g	at t	t ct	cga	t t c	768
Al a	Tyr	Tyr	Al a	Ser	Leu	Asp	Gl u	Gl u	Pro	Thr	Met	I l e	Ser	Arg	Phe	
			245						250					255		
ct t	gct	gaa	agt	gt c	t t g	cct	cat	caa	act	cgt	t at	t ac	t t c	t t t	gt t	816
Leu	Al a	Gl u	Ser	Val	Leu	Pro	Hi s	Gl n	Thr	Arg	Tyr	Tyr	Phe	Phe	Val	
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Leu	Gl y	Phe	Al a	Arg	Leu	Ser	Tr p	Al a	I l e	Gl n	Ser	Leu	Leu	Tyr	Ser	
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	290					295				300						
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Arg	Phe	Cys	Leu	Val	Ser	Hi s	Tr p	Thr	Leu	Phe	Thr	Tyr	Cys	Thr	Leu	
305					310					315					320	
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Al a	Tr p	Oys	Ser	Asn	Val	Tyr	Hi s	Met	I l e	Leu	Phe	Phe	Leu	Val	Ser	
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caa	gca	act	acc	ggt	t ac	aca	t t g	gcc	ct t	gt a	t t t	gct	t t g	aat	cac	1056
Gl n	Al a	Thr	Thr	Gl y	Tyr	Thr	Leu	Al a	Leu	Val	Phe	Al a	Leu	Asn	Hi s	
			340					345					350			
aat	ggt	at g	cct	gt g	at t	act	gaa	gaa	aag	gcc	gag	t ca	at g	gaa	t t t	1104
Asn	Gl y	Met	Pro	Val	I l e	Thr	Gl u	Gl u	Lys	Al a	Gl u	Ser	Met	Gl u	Phe	
			355				360					365				
t t c	gaa	at t	caa	gt g	at t	aca	ggt	cgt	gat	gt a	aca	ct c	t ct	cct	t t a	1152
Phe	Gl u	I l e	Gl n	Val	I l e	Thr	Gl y	Arg	Asp	Val	Thr	Leu	Ser	Pro	Leu	
	370					375						380				
ggt	gat	t g g	t t c	at g	ggt	gga	t t g	aac	t at	caa	at c	gag	cat	cat	gt t	1200
Gl y	Asp	Tr p	Phe	Met	Gl y	Gl y	Leu	Asn	Tyr	Gl n	I l e	Gl u	Hi s	Hi s	Val	
385					390					395					400	
t t c	cct	aat	at g	cct	cgt	cat	aat	t t a	cct	aaa	gt a	aag	cca	at g	gt c	1248
Phe	Pro	Asn	Met	Pro	Arg	Hi s	Asn	Leu	Pro	Lys	Val	Lys	Pro	Met	Val	
			405					410						415		
aag	t ca	ct t	t g t	aag	aaa	t at	gat	at t	aat	t at	cac	gat	act	gga	t t c	1296
Lys	Ser	Leu	Cys	Lys	Lys	Tyr	Asp	I l e	Asn	Tyr	Hi s	Asp	Thr	Gl y	Phe	
			420					425					430			
t t g	aaa	gga	aca	t t g	gaa	gt a	ct a	aag	aca	t t a	gat	at t	act	t cc	aag	1344
Leu	Lys	Gl y	Thr	Leu	Gl u	Val	Leu	Lys	Thr	Leu	Asp	I l e	Thr	Ser	Lys	
		435				440							445			
t t g	t ct	t t g	caa	t t g	agc	aaa	aag	t ca	t t t	t aa						1377
Leu	Ser	Leu	Gl n	Leu	Ser	Lys	Lys	Ser	Phe							
	450					455										

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 <213> Rhi zopus oryzae

<400> 16

PF58307.txt

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 1 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455

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PF58307. txt

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Met	Ser	Ser	Asp	Val	G y	Al a	Thr	Val	Pro	Hi s	Phe	Tyr	Thr	Arg	Al a	
1			5					10					15			
gaa	ttg	gct	gat	at c	cat	caa	gat	gt t	ct g	gac	aag	aaa	cct	gaa	gct	96
G u	Leu	Al a	Asp	l l e	Hi s	G n	Asp	Val	Leu	Asp	Lys	Lys	Pro	G u	Al a	
			20					25					30			
aga	aaa	ct a	att	gt c	gt c	gag	aat	aag	gt a	t ac	gat	at c	aca	gat	tt c	144
Arg	Lys	Leu	l l e	Val	Val	G u	Asn	Lys	Val	Tyr	Asp	l l e	Thr	Asp	Phe	
		35					40					45				
gt a	ttt	gat	cac	cct	ggc	ggt	gag	cga	gt a	ct t	ttg	act	caa	gag	ggc	192
Val	Phe	Asp	Hi s	Pro	G y	G y	G u	Arg	Val	Leu	Leu	Thr	G n	G u	G y	
	50				55						60					
aga	gac	gct	aca	gat	gt c	ttt	cat	gaa	at g	cat	cct	ccc	t ct	gct	t ac	240
Arg	Asp	Al a	Thr	Asp	Val	Phe	Hi s	G u	Met	Hi s	Pro	Pro	Ser	Al a	Tyr	
65					70				75						80	
gaa	ttg	ct g	gcg	aat	t gc	t at	gt t	ggc	gat	t gt	gag	ccc	aag	ct g	cct	288
G u	Leu	Leu	Al a	Asn	Cys	Tyr	Val	G y	Asp	Cys	G u	Pro	Lys	Leu	Pro	
				85					90					95		
at c	gat	t ca	acc	gat	aag	aag	gca	ttg	aat	t ct	gct	gct	ttt	gct	caa	336
l l e	Asp	Ser	Thr	Asp	Lys	Lys	Al a	Leu	Asn	Ser	Al a	Al a	Phe	Al a	G n	
			100					105					110			
gaa	att	cgt	gat	ct c	cga	gat	aaa	tt a	gaa	aaa	caa	ggc	t at	tt c	gac	384
G u	l l e	Arg	Asp	Leu	Arg	Asp	Lys	Leu	G u	Lys	G n	G y	Tyr	Phe	Asp	
			115				120					125				
gct	agt	act	ggt	tt c	t ac	at c	t at	aaa	gt t	t cc	act	acc	ct a	ct t	gt c	432
Al a	Ser	Thr	G y	Phe	Tyr	l l e	Tyr	Lys	Val	Ser	Thr	Thr	Leu	Leu	Val	
			130			135					140					
t gt	att	gt a	ggt	tt a	gct	at c	ct c	aaa	gct	t gg	ggt	aga	gag	t ct	act	480
Cys	l l e	Val	G y	Leu	Al a	l l e	Leu	Lys	Al a	Trp	G y	Arg	G u	Ser	Thr	
145				150						155					160	
ttg	gct	gt g	ttt	att	gct	t ct	tt a	gt t	gg t	ct t	ttt	t gg	cag	cag	528	
Leu	Al a	Val	Phe	l l e	Al a	Al a	Ser	Leu	Val	G y	Leu	Phe	Trp	G n	G n	
				165				170					175			
t gc	ggt	t gg	ct t	gcc	cat	gat	t at	gct	cac	t at	caa	gt c	at c	aaa	gac	576
Cys	G y	Trp	Leu	Al a	Hi s	Asp	Tyr	Al a	Hi s	Tyr	G n	Val	l l e	Lys	Asp	
			180					185					190			
ccc	aat	gt c	aat	aat	ct c	ttt	tt a	gt t	act	ttt	ggc	aac	ct g	gt t	caa	624
Pro	Asn	Val	Asn	Asn	Leu	Phe	Leu	Val	Thr	Phe	G y	Asn	Leu	Val	G n	
			195				200				205					
ggt	tt c	t ct	ct t	t ca	t gg	t gg	aaa	aac	aag	cac	aat	acc	cac	cac	gct	672
G y	Phe	Ser	Leu	Ser	Trp	Trp	Lys	Asn	Lys	Hi s	Asn	Thr	Hi s	Hi s	Al a	
			210			215					220					
agc	acc	aat	gt c	t ct	ggc	gaa	gat	ccc	gat	at c	gat	act	gcc	ccc	att	720
Ser	Thr	Asn	Val	Ser	G y	G u	Asp	Pro	Asp	l l e	Asp	Thr	Al a	Pro	l l e	
225					230					235				240		
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Leu	Leu	Trp	Asp	G u	Phe	Al a	Val	Al a	Asn	Phe	Tyr	G y	Ser	Leu	Lys	
				245					250				255			
gac	aat	gcc	agt	gga	ttt	gac	aga	tt c	att	gct	gaa	cac	att	tt g	ccc	816
Asp	Asn	Al a	Ser	G y	Phe	Asp	Arg	Phe	l l e	Al a	G u	Hi s	l l e	Leu	Pro	
			260					265					270			
t at	caa	act	cgt	t ac	t ac	tt c	tt c	att	ct t	ggt	tt c	gct	cgt	acc	t ct	864
Tyr	G n	Thr	Arg	Tyr	Tyr	Phe	Phe	l l e	Leu	G y	Phe	Al a	Arg	Thr	Ser	
			275				280						285			
t gg	gct	at c	caa	t ct	att	at c	t at	t cc	tt c	aag	aac	gaa	aca	tt g	aat	912
Trp	Al a	l l e	G n	Ser	l l e	l l e	Tyr	Ser	Phe	Lys	Asn	G u	Thr	Leu	Asn	
					295						300					
aaa	t ca	aag	ct c	tt g	t cc	t gg	t gt	gag	cgc	at c	ttt	tt g	att	gt t	cat	960
Lys	Ser	Lys	Leu	Leu	Ser	Trp	Cys	G u	Arg	l l e	Phe	Leu	l l e	Val	Hi s	
305					310					315					320	
t gg	gt c	ttt	tt c	act	t ac	t gc	act	att	gcc	t gg	at c	agc	t ct	at c	aga	1008
Trp	Val	Phe	Phe	Thr	Tyr	Cys	Thr	l l e	Al a	Trp	l l e	Ser	Ser	l l e	Arg	
				325					330					335		
aac	at c	gcc	at g	tt c	ttt	gt t	gt t	agc	caa	at c	act	act	ggt	t ac	tt g	1056
Asn	l l e	Al a	Met	Phe	Phe	Val	Val	Ser	G n	l l e	Thr	Thr	G y	Tyr	Leu	
			340					345						350		
ct c	gcc	at c	gt c	ttt	gct	at g	aac	cat	aat	ggc	at g	cct	gt t	t ac	agc	1104

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PF58307.txt

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 Gu Gu Ala Asn His Thr Gu Phe Tyr Gu Leu Gn Oys Ile Thr
 370 375 380
 ggt cgc gat gtc aac tgc act gta ttt ggc gat tgg ct c at g ggt gga 1200
 Gy Arg Asp Val Asn Cys Thr Val Phe Gy Asp Trp Leu Met Gy Gy 400
 385
 ttg aat tat caa att gag cac cat ct t ttc ccc gaa at g cct cga cat 1248
 Leu Asn Tyr Gn Ile Gu His His Leu Phe Pro Gu Met Pro Arg His
 405 410 415
 cat tta tcc aag gtg aaa tcc at g gt c aaa ccc at t gct caa aag tat 1296
 His Leu Ser Lys Val Lys Ser Met Val Lys Pro Ile Ala Gn Lys Tyr 430
 420 425 430
 aat atc cct tac cat gat acc aca gt c at t ggt ggt acc at t gaa gt c 1344
 Asn Ile Pro Tyr His Asp Thr Thr Val Ile Gy Gy Thr Ile Gu Val 445
 435 440 445
 ttg caa acc ttg gat ttt gtt caa aag at t t cg cag aaa ttc agc aaa 1392
 Leu Gn Thr Leu Asp Phe Val Gn Lys Ile Ser Gn Lys Phe Ser Lys 450 460
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 aaa at g ct c t aa 1404
 Lys Met Leu 465

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 <212> PRT
 <213> Mucor rouxi i

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 Gu Leu Ala Asp Ile His Gn Asp Val Leu Asp Lys Lys Pro Gu Ala
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 Arg Lys Leu Ile Val Val Gu Asn Lys Val Tyr Asp Ile Thr Asp Phe
 35 40 45
 Val Phe Asp His Pro Gy Gy Gu Arg Val Leu Leu Thr Gn Gu Gy
 50 55 60
 Arg Asp Ala Thr Asp Val Phe His Gu Met His Pro Pro Ser Ala Tyr
 65 70 75 80
 Gu Leu Leu Ala Asn Cys Tyr Val Gy Asp Cys Gu Pro Lys Leu Pro 95
 85 90
 Ile Asp Ser Thr Asp Lys Lys Ala Leu Asn Ser Ala Ala Phe Ala Gn
 100 105 110
 Gu Ile Arg Asp Leu Arg Asp Lys Leu Gu Lys Gn Gy Tyr Phe Asp
 115 120 125
 Ala Ser Thr Gy Phe Tyr Ile Tyr Lys Val Ser Thr Thr Leu Leu Val
 130 135 140
 Cys Ile Val Gy Leu Ala Ile Leu Lys Ala Trp Gy Arg Gu Ser Thr
 145 150 155 160
 Leu Ala Val Phe Ile Ala Ala Ser Leu Val Gy Leu Phe Trp Gn Gn
 165 170 175
 Cys Gy Trp Leu Ala His Asp Tyr Ala His Tyr Gn Val Ile Lys Asp
 180 185 190
 Pro Asn Val Asn Asn Leu Phe Leu Val Thr Phe Gy Asn Thr Leu Val Gn
 195 200 205
 Gy Phe Ser Leu Ser Trp Trp Lys Asn Lys His Asn Thr His His Ala
 210 215 220
 Ser Thr Asn Val Ser Gy Gu Asp Pro Asp Ile Asp Thr Ala Pro Ile
 225 230 235 240
 Leu Leu Trp Asp Gu Phe Ala Val Ala Asn Phe Tyr Gy Ser Leu Lys
 245 250 255
 Asp Asn Ala Ser Gy Phe Asp Arg Phe Ile Ala Gu His Ile Leu Pro
 260 265 270
 Tyr Gn Thr Arg Tyr Tyr Phe Phe Ile Leu Gy Phe Ala Arg Thr Ser
 275 280 285
 Trp Ala Ile Gn Ser Ile Ile Tyr Ser Phe Lys Asn Gu Thr Leu Asn
 290 295 300

PF58307.txt

Lys Ser Lys Leu Leu Ser Trp Cys G u 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 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 G u G u Ala Asn His Thr G u Phe Tyr G u Leu G n Cys Ile Thr
 370 375 380
 G y Arg Asp Val Asn Cys Thr Val Phe G y Asp Trp Leu Met G y G y
 385 390 395 400
 Leu Asn Tyr G n Ile G u His His Leu Phe Pro G u Met Pro Arg His
 405 410 415
 His Leu Ser Lys Val Lys Ser Met Val Lys Pro Ile Ala G n Lys Tyr
 420 425 430
 Asn Ile Pro Tyr His Asp Thr Thr Val Ile Gly Gly Thr Ile G u Val
 435 440 445
 Leu G n Thr Leu Asp Phe Val G n Lys Ile Ser G n Lys Phe Ser Lys
 450 455 460
 Lys Met Leu
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 Asn Ala G u Ala Leu Asn G u G y Lys Lys Asp Ala G u Ala Pro Phe
 20 25 30
 ct g at g at c at c gac aac aag gt g t ac gat gt c cgc gag t t c gt c cct 144
 Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg G u Phe Val Pro
 35 40 45
 gat cat ccc ggt gga agt gt g at t ct c acg cac gt t ggc aag gac ggc 192
 Asp His Pro G y G y Ser Val Ile Leu Thr His Val G y Lys Asp G y
 50 55 60
 act gac gt c t t t gac act t t t cac ccc gag gct gct t gg gag act ct t 240
 Thr Asp Val Phe Asp Thr Phe His Pro G u Ala Ala Trp G u Thr Leu
 65 70 75 80
 gcc aac t t t t ac gt t ggt gat at t gac gag agc gac cgc gat at c aag 288
 Ala Asn Phe Tyr Val G y Asp Ile Asp G u Ser Asp Arg Asp Ile Lys
 85 90 95
 aat gat gac t t t gcg gcc gag gt c cgc aag ct g cgt acc t t g t t c cag 336
 Asn Asp Asp Phe Ala Ala G u Val Arg Lys Leu Arg Thr Leu Phe G n
 100 105 110
 t ct ct t ggt t ac t ac gat t ct t cc aag gca t ac t ac gcc t t c aag gt c 384
 Ser Leu G y Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
 115 120 125
 t cg t t c aac ct c t gc at c t gg ggt t t g t cg acg gt c at t gt g gcc aag 432
 Ser Phe Asn Leu Cys Ile Trp G y Leu Ser Thr Val Ile Val Ala Lys
 130 135 140
 t gg ggc cag acc t cg acc ct c gcc aac gt g ct c t cg gct gcg ct t t t g 480
 Trp G y G n Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
 145 150 155 160
 ggt ct g t t c t gg cag ct g gga t gg t t g gct cac gac t t t t t g cat 528
 G y Leu Phe Trp G n G n Cys G y Trp Leu Ala His Asp Phe Leu His
 165 170 175
 cac cag gt c t t c cag gac cgt t t c t gg ggt gat ct t t t c ggc gcc t t c 576
 His G n Val Phe G n Asp Arg Phe Trp G y Asp Leu Phe G y Ala Phe

PF58307. txt

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Leu	Gly	Gly	Val	Cys	Gln	Gly	Phe	Ser	Ser	Ser	Trp	Trp	Lys	Asp	Lys		
		195					200					205					
c a c	a a c	a c t	c a c	c a c	g c c	g c c	c c c	a a c	g t c	c a c	g g c	g a a	g a t	c c c	g a c		672
His	Asn	Thr	His	His	Ala	Ala	Pro	Asn	Val	His	Gly	Glu	Asp	Pro	Asp		
	210					215					220						
a t t	g a c	a c c	c a c	c c t	c t g	t t g	a c c	t g g	a g t	g a g	c a t	g c g	t t g	g a g	a t g		720
Ile	Asp	Thr	His	Pro	Leu	Leu	Thr	Trp	Ser	Glu	His	Ala	Leu	Glu	Met		
	225				230					235				240			
t t c	t c g	g a t	g t c	c c a	g a t	g a g	g a g	c t g	a c c	c g c	a t g	t g g	t c g	c g t	t t c		768
Phe	Ser	Asp	Val	Pro	Asp	Glu	Glu	Leu	Thr	Arg	Met	Trp	Ser	Arg	Phe		
				245				250						255			
a t g	g t c	c t g	a a c	c a g	a c c	t g g	t t t	t a c	t t c	c c c	a t t	c t c	t c g	t t t	g c c		816
Met	Val	Leu	Asn	Gln	Thr	Trp	Phe	Tyr	Phe	Pro	Ile	Leu	Ser	Phe	Ala		
			260				265						270				
c g t	c t c	t c c	t g g	t g c	c t c	c a g	t c c	a t t	c t c	t t t	g t g	c t g	c c t	a a c	g g t		864
Arg	Leu	Ser	Trp	Oys	Leu	Gln	Ser	Ile	Leu	Phe	Val	Leu	Pro	Asn	Gly		
		275					280						285				
c a g	g c c	c a c	a a g	c c c	t c g	g g c	g c g	c g t	g t g	c c c	a t c	t c g	t t g	g t c	g a g		912
Gln	Ala	His	Lys	Pro	Ser	Gly	Ala	Arg	Val	Pro	Ile	Ser	Leu	Val	Glu		
	290					295				300							
c a g	c t g	t c g	c t t	g c g	a t g	c a c	t g g	a c c	t g g	t a c	c t c	g c c	a c c	a t g	t t c		960
Gln	Leu	Ser	Leu	Ala	Met	His	Trp	Thr	Trp	Tyr	Leu	Ala	Thr	Met	Phe		
	305				310					315					320		
c t g	t t c	a t c	a a g	g a t	c c c	g t c	a a c	a t g	c t g	g t g	t a c	t t t	t t g	g t g	t c g		1008
Leu	Phe	Ile	Lys	Asp	Pro	Val	Asn	Met	Leu	Val	Tyr	Phe	Leu	Val	Ser		
				325				330						335			
c a g	c c g	g t g	t g c	g g a	a a c	t t g	t t g	g c g	a t c	g t g	t t c	t c g	c t c	a a c	c a c		1056
Gln	Pro	Val	Cys	Gly	Asn	Leu	Leu	Ala	Ile	Val	Phe	Ser	Leu	Asn	His		
			340					345					350				
a a c	g g t	a t g	c c t	g t g	a t c	t c g	a a g	g a g	g c g	g t c	g a t	a t g	g a t	t t c			1104
Asn	Gly	Met	Pro	Val	Ile	Ser	Lys	Glu	Glu	Ala	Val	Asp	Met	Asp	Phe		
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t t c	a c g	a a g	c a g	a t c	a t c	a c g	g g t	c g t	g a t	g t c	c a c	c c g	g g t	c t a	t t t		1152
Phe	Thr	Lys	Gln	Ile	Ile	Thr	Gly	Arg	Asp	Val	His	Pro	Gly	Leu	Phe		
	370					375					380						
g c c	a a c	t g g	t t c	a c g	g g t	g g a	t t g	a a c	t a t	c a g	a t c	g a g	c a c	c a c	t t g		1200
Ala	Asn	Trp	Phe	Thr	Gly	Gly	Leu	Asn	Tyr	Gln	Ile	Glu	His	His	Leu		
	385				390				395						400		
t t c	c c t	t c g	a t g	c c t	c g c	c a c	a a c	t t t	t c a	a a g	a t c	c a g	c c t	g c t	g t c		1248
Phe	Pro	Ser	Met	Pro	Arg	His	Asn	Phe	Ser	Lys	Ile	Gln	Pro	Ala	Val		
				405				410						415			
g a g	a c c	c t g	t g c	a a a	a a g	t a c	a a t	g t c	c g a	t a c	c a c	a c c	a c c	g g t	a t g		1296
Glu	Thr	Leu	Cys	Lys	Lys	Tyr	Asn	Val	Arg	Tyr	His	Thr	Thr	Gly	Met		
			420					425					430				
a t c	g a g	g g a	a c t	g c a	g a g	g t c	t t t	a g c	c g t	c t g	a a c	g a g	g t c	t c c	a a g		1344
Ile	Glu	Gly	Thr	Ala	Glu	Val	Phe	Ser	Arg	Leu	Asn	Glu	Val	Ser	Lys		
		435					440					445					
g c t	g c c	t c c	a a g	a t g	g g c	a a g	g c a	c a g	t a a								1374
Ala	Ala	Ser	Lys	Met	Gly	Lys	Ala	Gln									
	450				455												

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 Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
 50 Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
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PF58307. txt

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85 90 95
Asn Asp Asp Phe Ala Ala Gl u Val Arg Lys Leu Arg Thr Leu Phe Gl n
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 Gl n Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145 150 155 160
Gly Leu Phe Trp Gl n Gl n Cys Gly Trp Leu Ala His Asp Phe Leu His
165 170 175
His Gl n Val Phe Gl n Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
180 185 190
Leu Gly Gly Val Cys Gl n 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 Gl u Asp Pro Asp
210 215 220
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Gl u His Ala Leu Gl u Met
225 230 235 240
Phe Ser Asp Val Pro Asp Gl u Gl u Leu Thr Arg Met Trp Ser Arg Phe
245 250 255
Met Val Leu Asn Gl n Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
260 265 270
Arg Leu Ser Trp Cys Leu Gl n Ser Ile Leu Phe Val Leu Pro Asn Gly
275 280 285
Gl n Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Gl u
290 295 300
Gl n 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
Gl n 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 Gl u Gl u Ala Val Asp Met Asp Phe
355 360 365
Phe Thr Lys Gl n Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
370 375 380
Al a Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gl n Ile Gl u His His Leu
385 390 395 400
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gl n Pro Ala Val
405 410 415
Gl u Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
420 425 430
Ile Gl u Gly Thr Ala Gl u Val Phe Ser Arg Leu Asn Gl u Val Ser Lys
435 440 445
Al a Ala Ser Lys Met Gly Lys Ala Gl n
450 455

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<211> 1377
<212> DNA
<213> Mbrt i e r e l l a a l p i n a

<220>
<221> CDS
<222> (1) . . (1377)

<400> 21
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Met Thr Thr Ser Asp Pro Ser Val Arg Ala Phe Thr Arg Ser Gl u Val
1 5 10 15
t t g cac gcc gat gcc t t g aac gag ggc aaa aag aac gcc gag gca ccg 96
Leu His Ala Asp Ala Leu Asn Gl u Gly Lys Lys Asn Ala Gl u Ala Pro
20 25 30
t t t ct c at g at c at c gac aac aag gt c t ac gat gt g cgc gag t t t at c 144
Phe Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Gl u Phe Ile
Sei t e 25

PF58307. txt

Leu	Phe	Pro	Ser	Met 405	Pro	Arg	His	His	Phe 410	Ser	Lys	Ile	Gln	Pro	Ala		
gtt	gaa	tcg	ctg	tgc	aag	aag	tac	ggg	gtc	cga	tac	cat	acg	acg	ggg		1296
Val	Gu	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	Gu	Val	Phe	Ser	Arg	Leu	Asn	Gly	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											

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 <212> PRT
 <213> Mbrtierella alpina

<400> 22

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Leu	His	Ala	Asp	Ala	Leu	Asn	Gu	Gly	Lys	Lys	Asn	Ala	Gly	Ala	Pro		
			20					25					30				
Phe	Leu	Met	Ile	Ile	Asp	Asn	Lys	Val	Tyr	Asp	Val	Arg	Gly	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	Gu	Thr	Phe	His	Pro	Gu	Ala	Ala	Trp	Gly	Thr		
65				70					75					80			
Leu	Ala	Asn	Phe	Tyr	Val	Gly	Asp	Ile	Val	Gly	Ser	Asp	Arg	Ala	Ile		
			85						90				95				
Gu	Asn	Asp	Gu	Phe	Ala	Ala	Gu	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	Gly	Asp	Pro		
	210					215					220						
Asp	Ile	Asp	Thr	His	Pro	Leu	Leu	Thr	Trp	Ser	Gly	His	Ala	Leu	Gly		
225				230					235					240			
Met	Phe	Ser	Asp	Val	Pro	Asp	Gu	Gu	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						
Gu	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	Gly	Gly	Ala	Val	Gly	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	Gly	His	His		
385					390					395				400			

PF58307. txt

Leu Phe Pro Ser Met Pro Arg His His Phe Ser Lys Ile Gl n Pro Ala
 405 Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly
 Val Gl u Ser Leu 420
 Met Ile Ala Ala Ser Lys Leu Gly Lys Ser Ala
 435 Gly Thr Ala Gl u Val Phe Ser Arg Leu Asn Val Ser
 440
 Gl n Ala Ala Ser Lys Leu Gly Lys Ser Ala
 450 Gly 455

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 <212> DNA
 <213> Phaeodactylum tricornutum

<220>
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 cgc aag at c agt tgg cag gaa gt c aag acc cac gcg t ct ccg gag gac 96
 Arg Lys Ile Ser Trp Gl n Gl u Val Lys Thr His Ala Ser Pro Gl u Asp
 20 25 30
 gcc t gg at c att cac t cc aat aag gt c t ac gac gt g t cc aac t gg 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 gt c att t t c acg cac gcc ggt gac gac at g 192
 Gl u His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
 50 55 60
 acg gac att t t c gct gcc t t t cac gca ccc gga t cg cag t cg ct c at g 240
 Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gl n Ser Leu Met
 65 70 75 80
 aag aag t t c t ac att ggc gaa t t g ct c ccg gaa acc acc ggc aag gag 288
 Lys Lys Phe Tyr Ile Gly Gl u Leu Leu Pro Gl u Thr Thr Gly Lys Gl u
 85 90 95
 ccg cag caa at c gcc t t t gaa aag ggc t ac cgc gat ct g cgc t cc aaa 336
 Pro Gl n Gl n Ile Ala Phe Gl u Lys Gly Tyr Arg Asp Leu Arg Ser Lys
 100 105 110
 ct c at c at g at g ggc at g t t c aag t cc aac aag t gg t t c t ac gt c t ac 384
 Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
 115 120 125
 aag t gc ct c agc aac at g gcc att t gg gcc gcc gcc t gt gct ct c gt c 432
 Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
 130 135 140
 t t t t ac t cg gac cgc t t c t gg gt a cac ct g gcc agc gcc gt c at g ct g 480
 Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
 145 150 155 160
 gga aca t t c t t t cag cag t cg gga t gg t t g gca cac gac t t t ct g cac 528
 Gly Thr Phe Phe Gl n Gl n Ser Gly Trp Leu Ala His Asp Phe Leu His
 165 170 175
 cac cag gt c t t c acc aag cgc aag cac ggg gat ct c gga gga ct c t t t 576
 His Gl n Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
 180 185 190
 t gg ggg aac ct c at g cag ggt t ac t cc gt a cag t gg t gg aaa aac aag 624
 Trp Gly Asn Leu Met Gl n Gly Tyr Ser Val Gl n Trp Trp Lys Asn Lys
 195 200 205
 cac aac gga cac cac gcc gt c ccc aac ct c cac t gc t cc t cc gca gt c 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 at c gat acc at g ccc ct t ct c gcc t gg 720
 Ala Gl n Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
 225 230 235 240
 t cc gt c cag caa gcc cag t ct t ac cgg gaa ct c caa gcc gac gga aag 768
 Ser Val Gl n Gl n Ala Gl n Ser Tyr Arg Gl u Leu Gl n Ala Asp Gly Lys
 245 250 255
 gat t cg ggt t t g gt c aag t t c at g at c cgt aac caa t cc t ac t t t t ac 816

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Asp	Ser	Gly	Leu	Val	Lys	Phe	Met	Ile	Arg	Asn	Gln	Ser	Tyr	Phe	Tyr		
260			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	tgc	ttc	ggc	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	ggg	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	tgc	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	t aa				1434
Glu	Phe	Val	Val	Asp	Phe	Val	Arg	Asp	Gly	Pro	Ala	Met					
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<210> 24
 <211> 477
 <212> PRT
 <213> Phaeodactylum tricorutum

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 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
 Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
 195 200
 His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
 210 215
 Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
 225 230 235
 Ser Val Gln Gln Ala Gln Ser Tyr Arg Gu 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 Gu Ser Phe
 275 280 285
 Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Gu Asn Ala Ala Leu Gu
 290 295 300
 Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Gu Lys Ala Gly Ile
 305 310 315
 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
 Gu Ser Phe Cys Lys Gu Trp Gly Val Gln Tyr His Gu Ala Asp Leu
 435 440 445
 Val Asp Gly Thr Met Gu Val Leu His His Leu Gly Ser Val Ala Gly
 450 455 460
 Gu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
 465 470 475

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 <212> DNA
 <213> Mbrtierella isabellina

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 Asn Ala Gu Ala Leu Asn Gu Gly Lys Lys Asp Ala Gu 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 Gu Phe Val Pro
 35 40 45
 gat cat ccc ggt gga agt gtt att ctg 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 ctg 240
 Thr Asp Val Phe Asp Thr Phe His Pro Gu Ala Ala Trp Gu 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 Gu Ser Asp Arg Ala Ile Lys

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      85
aac gat gac ttt gcc gcc gag gtc cgc aag ctg cgt act ttg ttc cag      336
Asn Asp Asp Phe Ala Ala Gu Val Arg Lys Leu Arg Thr Leu Phe Gn
      100
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
tca ttc aac ctg tgc atc tgg ggc ctg tcg acg ttc att gtt gcc aag      432
Ser Phe Asn Leu Cys lle Trp Gly Leu Ser Thr Thr lle Val Ala Lys
      130
tgg ggc cag acc tcg acc ctg gcc aac gtg ctg tcg gct gcg ctg ttg      480
Trp Gly Gn Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
      145
ggt ctg ttc tgg cag cag tgt gga tgg ttg gcg cac gac ttt ttg cac      528
Gly Leu Phe Trp Gn Gn Cys Gly Trp Leu Ala His Asp Phe Leu His
      165
cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc      576
His Gn Val Phe Gn Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
      180
ttg gga ggt gtc tgc cag ggc ttc tcg tcc tca tgg tgg aag gac aag      624
Leu Gly Gly Val Cys Gn Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
      195
cac aac act cac cac gcc gcc ccc aac gtg cac ggc gag gat ccc gac      672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Gu Asp Pro Asp
      210
att gac act cac cct ctg ttg acg tgg agt gag cat gct ttg gag atg      720
lle Asp Thr His Pro Leu Leu Thr Trp Ser Gu His Ala Leu Gu Met
      225
ttc tcg gac gtc cct gac gag gag ctg acc cgc atg tgg tcg cgc ttc      768
Phe Ser Asp Val Pro Asp Gu Gu Leu Thr Arg Met Trp Ser Arg Phe
      245
atg gtc ctt aac cag acc tgg ttc tac ttt ccc att ctg ttc gtc      816
Met Val Leu Asn Gn Thr Trp Phe Tyr Phe Pro lle Leu Ser Phe Ala
      260
cgt ctg tcc tgg tgc ctg cag tcc atc ctg ttt gtt ctg cct aac ggt      864
Arg Leu Ser Trp Cys Leu Gn Ser lle Leu Phe Val Leu Pro Asn Gly
      275
cag gcc cac aag ccc tct gga gcc cgt gtg ccc att tcc ttg gtc gag      912
Gn Ala His Lys Pro Ser Gly Ala Arg Val Pro lle Ser Leu Val Gu
      290
cag ctg tct ctt gcc gtg cac tgg acc tgg tac ctg gcc acc atg ttc      960
Gn Leu Ser Leu Ala Val His Trp Thr Trp Tyr Leu Ala Thr Met Phe
      305
ttg ttc att aag gac ccc gtg aac atg atg gtg tac ttt ttg gtg tct      1008
Leu Phe lle Lys Asp Pro Val Asn Met Met Val Tyr Phe Leu Val Ser
      325
cag gct gtt tgc ggt aac ctg ttg gcg att gtg ttc tcg ctg aac cac      1056
Gn Ala Val Cys Gly Asn Leu Leu Ala lle Val Phe Ser Leu Asn His
      340
aac ggt atg cct gtg atc tcc aag gag gaa gcc gtg gac atg gat ttc      1104
Asn Gly Met Pro Val lle Ser Lys Gu Gu Ala Val Asp Met Asp Phe
      355
ttc acc aag cag atc atc acg ggt cgt gat gtt cac cct ggt ctg ttc      1152
Phe Thr Lys Gn lle lle Thr Gly Arg Asp Val His Pro Gly Leu Phe
      370
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 Gn lle Gu His His Leu
      385
ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtg      1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys lle Gn Pro Ala Val
      405
gag acc ttg tgc aaa aag tat ggt gtc cga tac cac acc act ggc atg      1296
Gu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
      420
atc gag gga act gca gag gtc ttt agc cgt ttg aac gag gtc tcc aag      1344
lle Gu Gly Thr Ala Gu Val Phe Ser Arg Leu Asn Gu Val Ser Lys
      435
gcc gcc tcc aag atg ggt aag gcg cag taa
      440
      445

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 <212> PRT
 <213> Mbrtierella isabellina

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 20 25 30
 Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Gl u 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 Gl u Al a Trp Gl u Thr Leu
 65 70 75 80
 Al a Asn Phe Tyr Val Gly Asp Ile Asp Gl u Ser Asp Arg Al a Ile Lys
 85 90 95
 Asn Asp Asp Phe Al a Al a Gl u Val Arg Lys Leu Arg Thr Leu Phe G n
 100 105 110
 Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Al a Tyr Tyr Al a Phe Lys Val
 115 120 125
 Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Phe Ile Val Al a Lys
 130 135 140
 Trp Gly G n Thr Ser Thr Leu Al a Asn Val Leu Ser Al a Al a Leu Leu
 145 150 155 160
 Gly Leu Phe Trp G n G n Cys Gly Trp Leu Al a His Asp Phe Leu His
 165 170 175
 His G n Val Phe G n Asp Arg Phe Trp Gly Asp Leu Phe Gly Al a Phe
 180 185 190
 Leu Gly Gly Val Cys G n Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
 195 200 205
 His Asn Thr His His Al a Al a Pro Asn Val His Gly Gl u Asp Pro Asp
 210 215 220
 Ile Asp Thr His Pro Leu Leu Thr Trp Ser Gl u His Al a Leu Gl u Met
 225 230 235 240
 Phe Ser Asp Val Pro Asp Gl u Gl u Leu Thr Arg Met Trp Ser Arg Phe
 245 250 255
 Met Val Leu Asn G n Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Al a
 260 265 270
 Arg Leu Ser Trp Cys Leu G n Ser Ile Leu Phe Val Leu Pro Asn Gly
 275 280 285
 G n Al a His Lys Pro Ser Gly Al a Arg Val Pro Ile Ser Leu Val Gl u
 290 295 300
 G n Leu Ser Leu Al a Val His Trp Thr Trp Tyr Leu Al a 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
 G n Al a Val Cys Gly Asn Leu Leu Al a Ile Val Phe Ser Leu Asn His
 340 345 350
 Asn Gly Met Pro Val Ile Ser Lys Gl u Gl u Al a Val Asp Met Asp Phe
 355 360 365
 Phe Thr Lys G n Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
 370 375 380
 Al a Asn Trp Phe Thr Gly Gly Leu Asn Tyr G n Ile Gl u His His Leu
 385 390 395 400
 Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile G n Pro Al a Val
 405 410 415
 Gl u Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
 420 425 430
 Ile Gl u Gly Thr Al a Gl u Val Phe Ser Arg Leu Asn Gl u Val Ser Lys
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 Al a Al a Ser Lys Met Gly Lys Al a G n
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<210> 27
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 <222> (1)..(1374)

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 aat gcc gag gcc ct g aat gag ggc aag aag gat gcc gag gca cct t t c 96
 Asn Ala Gu Ala Leu Asn Gu Gy Lys Lys Asp Ala Gu Ala Pro Phe
 20 25 30
 t t g at g at c at c gac aac aag gt g t at gat gt c cgc gag t t t gt c cct 144
 Leu Met l l e l l e Asp Asn Lys Val Tyr Asp Val Arg Gu Phe Val Pro
 35 40 45
 gat cat ccc ggt gga agt gt t at t ct c acg cac gt t ggc aag gac ggc 192
 Asp Hi s Pro Gy Gy Ser Val l l e Leu Thr Hi s Val Gy Lys Asp Gy
 50 55 60
 act gac gt c t t t gac act t t c cac ccc gag gct gct t gg gag aca ct t 240
 Thr Asp Val Phe Asp Thr Phe Hi s Pro Gu Ala Ala Trp Gu Thr Leu
 65 70 75 80
 gcc aac t t t t ac gt t ggt gat at t gat gag agc gac cgt gcc at c aag 288
 Ala Asn Phe Tyr Val Gy Asp l l e Asp Gu Ser Asp Arg Ala l l e Lys
 85 90 95
 aac gat gac t t t gcg gcc gag gt c cgc aag ct g cgt act t t g t t c cag 336
 Asn Asp Asp Phe Ala Ala Gu Val Arg Lys Leu Arg Thr Leu Phe Gn
 100 105 110
 t ct ct g ggt t ac t ac gat t cc t cc aag gca t ac t ac gcc t t c aag gt c 384
 Ser Leu Gy Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
 115 120 125
 t ca t t c aac ct c t gc at c t gg ggc ct g t cg acg t t c at t gt t gcc aag 432
 Ser Phe Asn Leu Cys l l e Trp Gy Leu Ser Thr Phe l l e Val Ala Lys
 130 135 140
 t gg ggc cag acc t cg acc ct c gcc aac gag ct c t cg gct gcg ct c t t g 480
 Trp Gy Gn Thr Ser Thr Leu Ala Asn Gu Leu Ser Ala Ala Leu Leu
 145 150 155 160
 ggt ct c t t c t gg cag cag aga gga t gg t t g gcg cac gac t t t t t g cac 528
 Gy Leu Phe Trp Gn Gn Arg Gy Trp Leu Ala Hi s Asp Phe Leu Hi s
 165 170 175
 cac cag gt c t t c cag gac cgt t t c t gg gga gat ct t t t c ggc gcc t t c 576
 Hi s Gn Val Phe Gn Asp Arg Phe Trp Gy Asp Leu Phe Gy Ala Phe
 180 185 190
 t t g gga gga gac t gc cag gcc t t c t cg t cc t ca t gg t gg aag gac aag 624
 Leu Gy Gy Asp Cys Gn Gy Phe Ser Ser Ser Trp Trp Lys Asp Lys
 195 200 205
 cac aac act cac cac gcc gcc ccc aac gt c cac ggc gag gat ccc gac 672
 Hi s Asn Thr Hi s Hi s Ala Ala Pro Asn Val Hi s Gy Gu Asp Pro Asp
 210 215 220
 at t gac act cac cct ct g t t g acg t gg agt gag cat gct t t g gag at g 720
 l l e Asp Thr Hi s Pro Leu Leu Thr Trp Ser Gu Hi s Ala Leu Gu Met
 225 230 235 240
 t t c t cg gac gt c cct gac gag gag ct g acc cgc at g t gg t cg cgc t t c 768
 Phe Ser Asp Val Pro Asp Gu Gu Leu Thr Arg Met Trp Ser Arg Phe
 245 250 255
 at g gt c ct t aac cag acc t gg t t c t ac t t t ccc at t ct c t cg t t t gcc 816
 Met Val Leu Asn Gn Thr Trp Phe Tyr Phe Pro l l e Leu Ser Phe Ala
 260 265 270
 cgt ct c t cc t gg t gc ct c cag t cc at c ct c t t t gt t ct g cct aac ggt 864
 Arg Leu Ser Trp Cys Leu Gn Ser l l e Leu Phe Val Leu Pro Asn Gy
 275 280 285
 cag gcc cac aag ccc t ct gga gcc cgt gt g ccc at t t cc t t g gt c gag 912
 Gn Ala Hi s Lys Pro Ser Gy Ala Arg Val Pro l l e Ser Leu Val Gu
 290 295 300
 cag ct g t ct ct t gcc at g cac t gg acc t gg t ac ct c gcc acc at g t t c 960

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G n	Leu	Ser	Leu	Al a	Met	Hi s	Tr p	Thr	Tr p	Tyr	Leu	Al a	Thr	Met	Phe		
305					310					315					320		
t t g	t t c	a t t	a a g	g a c	c c c	g t c	a a c	a t g	a t g	g t g	t a c	t t t	t t g	g t g	t c t		1008
Leu	Phe	I l e	Lys	Asp	Pro	Val	Asn	Met	Met	Val	Tyr	Phe	Leu	Val	Ser		
				325						330				335			
c a g	g c t	g t t	t g c	g g t	a a c	c t g	t t g	g c g	a t t	g t g	t t c	t c g	c t c	a a c	c a c		1056
G n	Al a	Val	Cys	G y	Asn	Leu	Leu	Al a	I l e	Val	Phe	Ser	Leu	Asn	Hi s		
			340					345					350				
a a c	g g t	a t g	c c t	g t g	a t c	t c c	a a g	g a g	g a a	g c c	g t c	g a c	a t g	g a t	t t c		1104
Asn	G y	Met	Pro	Val	I l e	Ser	Lys	G u	G u	Al a	Val	Asp	Met	Asp	Phe		
		355					360					365					
t t c	a c c	a a g	c a g	a t c	a t c	a c g	g g t	c g t	g a t	g t t	c a c	c c t	g g t	c t g	t t c		1152
Phe	Thr	Lys	G n	I l e	I l e	Thr	G y	Arg	Asp	Val	Hi s	Pro	G y	Leu	Phe		
	370					375					380						
g c c	a a c	t g g	t t c	a c g	g g t	g g a	t t g	a a c	t a c	c a g	a t t	g a g	c a c	c a c	t t g		1200
Al a	Asn	Tr p	Phe	Thr	G y	G y	Leu	Asn	Tyr	G n	I l e	G u	Hi s	Hi s	Leu		
385				390						395					400		
t t c	c c t	t c g	a t g	c c t	c g c	c a c	a a c	t t t	t c a	a a g	a t c	c a g	c c t	g c t	g t c		1248
Phe	Pro	Ser	Met	Pro	Arg	Hi s	Asn	Phe	Ser	Lys	I l e	G n	Pro	Al a	Val		
				405				410						415			
g a g	a c c	t t g	t g c	a a a	a a g	t a t	g g t	g t c	c g a	t a c	c a c	a c c	a c t	g g c	a t g		1296
G u	Thr	Leu	Cys	Lys	Lys	Tyr	G y	Val	Arg	Tyr	Hi s	Thr	Thr	G y	Met		
			420				425						430				
a t c	g a g	g g a	a c t	g c a	g a g	g t c	t t t	a g c	c g t	t t g	a a c	g a g	g t c	t c c	a a g		1344
I l e	G u	G y	Thr	Al a	G u	Val	Phe	Ser	Arg	Leu	Asn	G u	Val	Ser	Lys		
		435				440						445					
g c c	g c c	t c c	a a g	a t g	g g t	a a g	g c g	c a g	t a a								1374
Al a	Al a	Ser	Lys	Met	G y	Lys	Al a	G n									
	450				455												

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 <212> PRT
 <213> Mbr t i e r e l l a a l p i n a

<400> 28

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1				5					10					15			
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			20					25					30				
Leu	Met	I l e	Asp	Asn	Lys	Val	Tyr	Asp	Val	Arg	G u	Phe	Val	Pro			
		35				40						45					
Asp	Hi s	Pro	G y	G y	Ser	Val	I l e	Leu	Thr	Hi s	Val	G y	Lys	Asp	G y		
	50			55						60							
Thr	Asp	Val	Phe	Asp	Thr	Phe	Hi s	Pro	G u	Al a	Al a	Tr p	G u	Thr	Leu		
65				70						75				80			
Al a	Asn	Phe	Tyr	Val	G y	Asp	I l e	Asp	G u	Ser	Asp	Arg	Al a	I l e	Lys		
				85				90						95			
Asn	Asp	Asp	Phe	Al a	Al a	G u	Val	Arg	Lys	Leu	Arg	Thr	Leu	Phe	G n		
			100					105					110				
Ser	Leu	G y	Tyr	Tyr	Asp	Ser	Ser	Lys	Al a	Tyr	Tyr	Al a	Phe	Lys	Val		
		115				120						125					
Ser	Phe	Asn	Leu	Cys	I l e	Tr p	G y	Leu	Ser	Thr	Phe	I l e	Val	Al a	Lys		
	130					135					140						
Tr p	G y	G n	Thr	Ser	Thr	Leu	Al a	Asn	G u	Leu	Ser	Al a	Al a	Leu	Leu		
145				150						155				160			
G y	Leu	Phe	Tr p	G n	G n	Arg	G y	Tr p	Leu	Al a	Hi s	Asp	Phe	Leu	Hi s		
				165				170						175			
Hi s	G n	Val	Phe	G n	Asp	Arg	Phe	Tr p	G y	Asp	Leu	Phe	G y	Al a	Phe		
			180					185					190				
Leu	G y	G y	Asp	Cys	G n	G y	Phe	Ser	Ser	Ser	Tr p	Tr p	Lys	Asp	Lys		
		195				200					205						
Hi s	Asn	Thr	Hi s	Hi s	Al a	Al a	Pro	Asn	Val	Hi s	G y	G u	Asp	Pro	Asp		
	210					215					220						
I l e	Asp	Thr	Hi s	Pro	Leu	Leu	Thr	Tr p	Ser	G u	Hi s	Al a	Leu	G u	Met		
225				230						235				240			
Phe	Ser	Asp	Val	Pro	Asp	G u	G u	Leu	Thr	Arg	Met	Tr p	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 Gu
 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 Gu Gu 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 Gu 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
 Gu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
 420 425 430
 Ile Gu Gly Thr Ala Gu Val Phe Ser Arg Leu Asn Gu 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
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 1 5 10 15
 atc cgc gag cac gcg acg ccc gcg acc cgc tgg atc gtg att cac cac 96
 Ile Arg Gu 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 tgc 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 ctg acg cag gcc ggc gag gac gcc acg gac gcc ttc gcg gtc ttc 192
 Met Leu Thr Gln Ala Gly Gu Asp Ala Thr Asp Ala Phe Ala Val Phe
 50 55 60
 cac ccg tcc tgc gcg ctg aag ctg ctg gag cag ttc tac gtc ggc gac 240
 His Pro Ser Ser Ala Leu Lys Leu Leu Gu 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 Gu Thr Ser Lys Ala Gu Ile Gu Gly Gu Pro Ala Ser Asp
 85 90 95
 gag gag cgc gcg cgc cgc gag cgc atc aac gag ttc atc gcg tcc tac 336
 Gu Gu Arg Ala Arg Arg Gu Arg Ile Asn Gu Phe Ile Ala Ser Tyr
 100 105 110
 cgt cgt ctg cgc gtc aag gtc aag ggc atg ggg ctg 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 ctg tac tac gcg tgg aag ctg 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
 ctg tgc 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 ctg ttc tac cag cag tcc gga tgg ctg 528

PF58307.txt

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 ctg 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 ctg 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
 tgc 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 ctg gcg cgc ctg agc tgg ctg gcg cag tgc 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 tgc 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 ctg 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 ctg atg ggc cag gcg tcc tgc ggc ttg ctg ctg gcg ctg gtc 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 tgc gtg tac gag cgc gaa acc aag ccg 1104
 Ser Ile Gly His Asn Gly Met Ser Val Tyr Glu Arg Gln Thr Lys Pro
 355 360 365
 gac ttc tgg cag ctg cag gtg acc acg acg cgc aac atc cgc gcg tgc 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 ctg 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
 ctg atc aag tgc 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

<210> 30
 <211> 459
 <212> PRT
 <213> Pythium irregularis

<400> 30
 Met Val Asp Leu Lys Pro Gly Val Lys Arg Leu Val Ser Trp Lys Glu
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 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
 Sei te 36

PF58307. txt

Met Leu Thr 35 Ala Gly Gu 40 Asp Ala Thr Asp Ala Phe Ala Val Phe
 50
 His Pro Ser Ser Ala Leu Lys Leu Leu Gu Gn Phe Tyr Val Gly Asp
 65 70 75 80
 Val Asp Gu Thr Ser Lys Ala Gu Ile Gu Gly Gu Pro Ala Ser Asp
 85 90 95
 Gu Gu Arg Ala Arg Arg Gu Arg Ile Asn Gu Phe Ile Ala Ser Tyr
 100 105
 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 Gn Gn Ser Gly Trp Leu
 165 170 175
 Ala His Asp Phe Leu His Asn Gn Val Cys Gu Asn Arg Thr Leu Gly
 180 185 190
 Asn Leu Ile Gly Cys Leu Val Gly Asn Ala Trp Gn Gly Phe Ser Val
 195 200 205
 Gn Trp Trp Lys Asn Lys His Asn Leu His His Ala Val Pro Asn Leu
 210 215 220
 His Ser Ala Lys Asp Gu Gly Phe Ile Gly Asp Pro Asp Ile Asp Thr
 225 230 235 240
 Met Pro Leu Leu Ala Trp Ser Lys Gu Met Ala Arg Lys Ala Phe Gu
 245 250 255
 Ser Ala His Gly Pro Phe Phe Ile Arg Asn Gn Ala Phe Leu Tyr Phe
 260 265 270
 Pro Leu Leu Leu Leu Ala Arg Leu Ser Trp Leu Ala Gn Ser Phe Phe
 275 280 285
 Tyr Val Phe Thr Gu Phe Ser Phe Gly Ile Phe Asp Lys Val Gu Phe
 290 295 300
 Asp Gly Pro Gu Lys Ala Gly Leu Ile Val His Tyr Ile Trp Gn Leu
 305 310 315 320
 Ala Ile Pro Tyr Phe Cys Asn Met Ser Leu Phe Gu Gly Val Ala Tyr
 325 330 335
 Phe Leu Met Gly Gn Ala Ser Cys Gly Leu Leu Leu Ala Leu Val Phe
 340 345 350
 Ser Ile Gly His Asn Gly Met Ser Val Tyr Gu Arg Gu Thr Lys Pro
 355 360 365
 Asp Phe Trp Gn Leu Gn 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 Gn 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 Gu Phe Asp Ile Pro Phe His Gu Thr
 420 425 430
 Gly Phe Trp Gu Gly Ile Tyr Gu Val Val Asp His Leu Ala Asp Ile
 435 440 445
 Ser Lys Gu Phe Ile Thr Gu Phe Pro Ala Met
 450 455

<210> 31
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 <213> Mbr t i e r e l l a a l p i n a

<220>
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 <222> (1) . . (1374)

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 aat gcc gag gcc ct g aat gag ggc aag aag gat gcc gag gca cct t t c 96
 Sei t e 37

PF58307. txt

Asn	Ala	Glu	Ala	Leu	Asn	Glu	Gly	Lys	Lys	Asp	Ala	Glu	Ala	Pro	Phe		
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	tgg	gag	aca	ctt			240
Thr	Asp	Val	Phe	Asp	Thr	Phe	His	Pro	Glu	Ala	Ala	Trp	Glu	Thr	Leu		80
	65				70				75								
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		160
	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		240
	225				230					235							
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										
cag	gcc	cac	aag	ccc	tct	gga	ggc	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		320
	305				310					315							
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		
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gcc	aac	tgg	ttc	acg	ggg	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	ggg	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	ggg	aag	gcg	cag	taa							1374
Ala	Ala	Ser	Lys	Met	Gly	Lys	Ala	Gln								
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			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
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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
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His	Gln	Val	Phe	Gln	Asp	Arg	Phe	Trp	Gly	Asp	Leu	Phe	Gly	Ala	Phe
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Leu	Gly	Gly	Asp	Cys	Gln	Gly	Phe	Ser	Ser	Ser	Trp	Trp	Lys	Asp	Lys
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His	Asn	Thr	His	His	Ala	Ala	Pro	Asn	Val	His	Gly	Glu	Asp	Pro	Asp
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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 G n I l e I l e Thr G y Arg Asp Val H i s Pro G y Leu Phe
 370 375 380
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 385 390 395 400
 P h e P r o S e r M e t P r o A r g H i s A s n P h e S e r L y s I l e G l n P r o A l a V a l
 405 410 415
 G u Thr Leu C y s L y s L y s T y r G y V a l A r g T y r H i s Thr Thr G y M e t
 420 425 430
 I l e G u G y Thr A l a G u V a l P h e S e r A r g Leu A s n G u V a l S e r L y s
 435 440 445
 A l a A l a S e r L y s M e t G y L y s A l a G n
 450 455

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 M e t A l a A l a A l a P r o S e r V a l A r g T h r P h e T h r A r g A l a G u I l e L e u
 1 5 10 15
 a a t g c c g a g g c c c t g a a t g a g g g c a a g g a t g c c g a g g c a c c t t t c 96
 A s n A l a G l u A l a L e u A s n G u G y L y s L y s A s p A l a G u A l a P r o P h e
 20 25 30
 t t g a t g a t c a t c g a c a a c a a g g t g t a t g a t g t c c g c g a g t t t g t c c c t 144
 L e u M e t I l e I l e A s p A s n L y s V a l T y r A s p V a l A r g G l u P h e V a l P r o
 35 40 45
 g a t c a t c c c g g t g g a a g t g t a t t c t c a c g c a c g t t g g c a a g g a c g g c 192
 A s p H i s P r o G y G y S e r V a l I l e L e u T h r H i s V a l G y L y s A s p G y
 50 55 60
 a c t g a c g t c t t t g a c a c t t t c c a c c c c g a g g c t t g g g a g a c a c t t 240
 T h r A s p V a l P h e A s p T h r P h e H i s P r o G l u A l a T r p G l u T h r L e u
 65 70 75 80
 g c c a a c t t t t a t g t t g g t g a t a t t g a t g a g a g c g a c c g t g c c a t c a a a 288
 A l a A s n P h e T y r V a l G y A s p I l e A s p G l u S e r A s p A r g A l a I l e L y s
 85 90 95
 a a c g a t g a c t t t g c g g c c g a g g t c c g c a a g c t g c g t a c t t t g t t c c a g 336
 A s n A s p A s p P h e A l a A l a G u V a l A r g L y s L e u A r g T h r L e u P h e G l n
 100 105 110
 t c t c t g g g t t a c t a c g a t t c c t c c a a g g c a t a c t a c g c c t t c a a g g t c 384
 S e r L e u G y T y r T y r A s p S e r S e r L y s A l a T y r T y r 125 P h e L y s V a l
 115 120 125
 t c a t t c a a c c t c t g c a t c t g g g g c c t g t c g a c g t t c a t t g t t g c c a a g 432
 S e r P h e A s n L e u C y s I l e T r p G y L e u S e r T h r P h e I l e V a l A l a L y s
 130 135 140
 t g g g g c c a g a c c t c g a c c c t c g c c a a c g t a c t c t c g g t g c g c t c t t g 480
 T r p G y G l n T h r S e r T h r L e u A l a A s n V a l L e u S e r A l a A l a L e u L e u
 145 150 155 160
 g g t c t c t t c t g g c a g c a g t g t g g a t g g t t t g g c g c a c g a c t t t t t g c a c 528
 G y L e u P h e T r p G l n G l n C y s G y T r p L e u A l a H i s A s p P h e L e u H i s
 165 170 175
 c a c c a g g t c t t c c a g g a c c g t t t c t g g g g t g a t c t t t t c g g c g c c t t c 576
 H i s G l n V a l P h e G l n A s p A r g P h e T r p G y A s p L e u P h e G y A l a P h e
 180 185 190
 t t g g g a g g t g t c t g c c a g g g c t t c t c g t c c t c a t g g t g g a a g g a c a a g 624
 L e u G y G y V a l C y s G l n G y P h e S e r S e r S e r T r p T r p L y s A s p L y s
 195 200 205
 c a c a a c a c t c a c c a c g c c g c c c c a a c g t c c a c g g c g a g g a t c c c g a c 672
 H i s A s n T h r H i s H i s A l a A l a P r o A s n V a l H i s G y G l u A s p P r o A s p
 210 215 220
 a t t g a c a c t c a c c c t c t g t t g a c g t g g a g t g a g c a t g c t t t g g a g a t g 720
 I l e A s p T h r H i s P r o L e u L e u T h r T r p S e r G l u H i s A l a L e u G u M e t
 225 230 235 240

PF58307. txt

t t c	t c g	g a c	g t c	c c t	g a c	g a g	g a g	c t g	a c c	c g c	a t g	t g g	t c g	c g c	t t c	768
P h e	S e r	A s p	V a l	P r o	A s p	G u	G u	L e u	T h r	A r g	M e t	T r p	S e r	A r g	P h e	
				245					250					255		
a t g	g t c	c t t	a a c	c a g	a c c	t g g	t t c	t a c	t t t	c c c	a t t	c t c	t c g	t t t	g c c	816
M e t	V a l	L e u	A s n	G n	T h r	T r p	P h e	T y r	P h e	P r o	I l e	L e u	S e r	P h e	A l a	
			260					265					270			
c g t	c t c	t c c	t g g	t g c	c t c	c a g	t c c	a t c	c t c	c t t	g t t	c t g	c c t	a a c	g g t	864
A r g	L e u	S e r	T r p	C y s	L e u	G n	S e r	I l e	L e u	L e u	V a l	L e u	P r o	A s n	G y	
		275					280						285			
c a g	g c c	c a c	a a g	c c c	t c t	g g a	g c c	c g t	g t g	t c c	a t t	t c c	t t g	g t c	g a g	912
G n	A l a	H i s	L y s	P r o	S e r	G y	A l a	A r g	V a l	S e r	I l e	S e r	L e u	V a l	G u	
	290					295				300						
c a g	c t g	t c t	c t t	g c c	a t g	c a c	t g g	a c c	t g g	t a c	c t c	g c c	a c c	a t g	t t c	960
G n	L e u	S e r	L e u	A l a	M e t	H i s	T r p	T h r	T r p	T y r	L e u	A l a	T h r	M e t	P h e	
	305				310					315					320	
t t g	t t c	a t t	a a g	g a c	c c c	g t c	a a c	a t g	a t g	g t g	t a c	t t t	t t g	g t g	t c t	1008
L e u	P h e	I l e	L y s	A s p	P r o	V a l	A s n	M e t	M e t	V a l	T y r	P h e	L e u	V a l	S e r	
				325					330					335		
c a g	g c t	g t t	t g c	g g t	a a c	c t g	t t g	g c g	a t t	g t g	t t c	t c g	c t c	a a c	c a c	1056
G n	A l a	V a l	C y s	G y	A s n	L e u	L e u	A l a	I l e	V a l	P h e	S e r	L e u	A s n	H i s	
			340					345					350			
a a c	g g t	a t g	c c t	g t g	a t c	t c c	a a g	g a g	g a a	g c c	g t c	g a c	a t g	g a t	t t c	1104
A s n	G y	M e t	P r o	V a l	I l e	S e r	L y s	G u	G u	A l a	V a l	A s p	M e t	A s p	P h e	
		355					360					365				
t t c	a c c	a a g	c a g	a t c	a t c	a c g	g g t	c g t	g a t	g t t	c a c	c c t	g g t	c t g	t t c	1152
P h e	T h r	L y s	G n	I l e	I l e	T h r	G y	A r g	A s p	V a l	H i s	P r o	G y	L e u	P h e	
	370					375					380					
g c c	a a t	t g g	t t c	a c g	g g t	g g a	t t g	a a c	t a c	c a g	a t t	g a g	c a c	c a c	t t g	1200
A l a	A s n	T r p	P h e	T h r	G y	G y	L e u	A s n	T y r	G n	I l e	G u	H i s	H i s	L e u	
	385				390					395					400	
t t c	c c t	t c g	a t g	c c t	c g c	c a c	a a c	t t t	t c a	a a g	a t c	c a g	c c t	g c t	g t c	1248
P h e	P r o	S e r	M e t	P r o	A r g	H i s	A s n	P h e	S e r	L y s	I l e	G n	P r o	A l a	V a l	
				405					410					415		
g a g	a c c	t t g	t g c	a a a	a a g	t a t	g g t	g t c	c g a	t a c	c a c	a c c	a c t	g g c	a t g	1296
G u	T h r	L e u	C y s	L y s	L y s	T y r	G y	V a l	A r g	T y r	H i s	T h r	T h r	G y	M e t	
			420					425					430			
a t c	g a g	g g a	a c t	g c a	g a g	g t c	t t t	a g c	c g t	t t g	a a c	g a g	g t c	t c c	a a g	1344
I l e	G u	G y	T h r	A l a	G u	V a l	P h e	S e r	A r g	L e u	A s n	G u	V a l	S e r	L y s	
		435					440					445				
g c c	g c c	t c c	a a g	a t g	g g t	a a g	g c g	c a g	t a a							1374
A l a	A l a	S e r	L y s	M e t	G y	L y s	A l a	G n								
	450					455										

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 20 25 30
 L e u M e t I l e I l e A s p A s n L y s V a l T y r A s p V a l A r g G u P h e V a l P r o
 35 40 45
 A s p H i s P r o G y G y S e r V a l I l e L e u T h r H i s V a l G y L y s A s p G y
 50 55 60
 T h r A s p V a l P h e A s p T h r P h e H i s P r o G u A l a A l a T r p G u T h r L e u
 65 70 75 80
 A l a A s n P h e T y r V a l G y A s p I l e A s p G u S e r A s p A r g A l a I l e L y s
 85 90 95
 A s n A s p A s p P h e A l a A l a G u V a l A r g L y s L e u A r g T h r L e u P h e G n
 100 105 110
 S e r L e u G y T y r T y r A s p S e r S e r L y s A l a T y r T y r A l a P h e L y s V a l
 115 120 125
 S e r P h e A s n L e u C y s I l e T r p G y L e u S e r T h r P h e I l e V a l A l a L y s
 130 135 140
 T r p G y G n T h r S e r T h r L e u A l a A s n V a l L e u S e r A l a A l a L e u L e u
 S e i t e 41

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 180 185 190
 Leu G y G y Val Cys G n G y Phe Ser Ser Ser Trp Trp Lys Asp Lys
 195 200 205
 Hi s Asn Thr Hi s Hi s Al a Al a Pro Asn Val Hi s G y G u Asp Pro Asp
 210 215 220
 Ile Asp Thr Hi s Pro Leu Leu Thr Trp Ser G u Hi s Al a Leu G u Met
 225 230 235 240
 Phe Ser Asp Val Pro Asp G u G u Leu Thr Arg Met Trp Ser Arg Phe
 245 250 255
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 260 265 270
 Arg Leu Ser Trp Cys Leu G n Ser Ile Leu Leu Val Leu Pro Asn G y
 275 280 285
 G n Al a Hi s Lys Pro Ser G y Al a Arg Val Ser Ile Ser Leu Val G u
 290 295 300
 G n Leu Ser Leu Al a Met Hi s Trp Thr Trp Tyr Leu Al a 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
 G n Al a Val Cys G y Asn Leu Leu Al a Ile Val Phe Ser Leu Asn Hi s
 340 345 350
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 355 360 365
 Phe Thr Lys G n Ile Ile Thr G y Arg Asp Val Hi s Pro G y Leu Phe
 370 375 380
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 385 390 395 400
 Phe Pro Ser Met Pro Arg Hi s Asn Phe Ser Lys Ile G n Pro Al a Val
 405 410 415
 G u Thr Leu Cys Lys Lys Tyr G y Val Arg Tyr Hi s Thr Thr G y Met
 420 425 430 435
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 Ile Asp Val G u Hi s Leu Al a Thr Met Pro Leu Val Ser Asp Phe Leu
 20 25 30
 aat gt c ct g gga acg act ttg ggc cag t gg agt ct t t cc act aca t t c 144
 Asn Val Leu G y Thr Thr Leu G y G n Trp Ser Leu Ser Thr Thr Phe
 35 40 45
 gct t t c aag agg ct c acg act aag aaa cac agt t cg gac at c t cg gt g 192
 Al a Phe Lys Arg Leu Thr Thr Lys Lys Hi s Ser Ser Asp Ile Ser Val
 50 55 60
 gag gca caa aaa gaa t cg gt t gcg cgg ggg cca gt t gag aat at t t ct 240
 G u Al a G n Lys G u Ser Val Al a Arg G y Pro Val G u Asn Ile Ser
 65 70 75 80
 caa t cg gt t gcg cag ccc at c agg cgg agg t gg gt g cag gat aaa aag 288
 G n Ser Val Al a G n Pro Ile Arg Arg Arg Trp Val G n Asp Lys Lys
 85 90 95

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ccg	gt t	act	t ac	agc	ct g	aag	gat	gt a	gct	t cg	cac	gat	at g	ccc	cag	336
Pro	Val	Thr	Tyr	Ser	Leu	Lys	Asp	Val	Al a	Ser	Hi s	Asp	Met	Pro	G n	
			100					105					110			
gac	t gc	t gg	at t	at a	at c	aaa	gag	aag	gt g	t at	gat	gt g	agc	acc	t t c	384
Asp	Cys	Trp	I le	I le	I le	Lys	G u	Lys	Val	Tyr	Asp	Val	Ser	Thr	Phe	
		115					120					125				
gct	gag	cag	cac	cct	gga	ggc	acg	gt t	at c	aac	acc	t ac	t t c	gga	cga	432
Al a	G u	G n	Hi s	Pro	G y	G y	Thr	Val	I le	Asn	Thr	Tyr	Phe	G y	Arg	
		130				135					140					
gac	gcc	aca	gat	gt t	t t c	t ct	act	t t c	cac	gca	t cc	acc	t ca	t gg	aag	480
Asp	Al a	Thr	Asp	Val	Phe	Ser	Thr	Phe	Hi s	Al a	Ser	Thr	Ser	Trp	Lys	
145					150				155					160		
at t	ct t	cag	aat	t t c	t ac	at c	ggg	aac	ct t	gt t	agg	gag	gag	ccg	act	528
I le	Leu	G n	Asn	Phe	Tyr	I le	G y	Asn	Leu	Val	Arg	G u	G u	Pro	Thr	
			165					170						175		
t t g	gag	ct g	ct g	aag	gag	t ac	aga	gag	t t g	aga	gcc	ct t	t t c	t t g	aga	576
Leu	G u	Leu	Leu	Lys	G u	Tyr	Arg	G u	Leu	Arg	Al a	Leu	Phe	Leu	Arg	
			180					185					190			
gaa	cag	ct t	t t c	aag	agt	t cc	aaa	t cc	t ac	t ac	ct t	t t c	aag	act	ct c	624
G u	G n	Leu	Phe	Lys	Ser	Ser	Lys	Ser	Tyr	Tyr	Leu	Phe	Lys	Thr	Leu	
		195				200					205					
at a	aat	gt t	t cc	at t	gt t	gcc	aca	agc	at t	gcg	at a	at c	agt	ct g	t ac	672
I le	Asn	Val	Ser	I le	Val	Al a	Thr	Ser	I le	Al a	I le	I le	Ser	Leu	Tyr	
	210					215				220						
aag	t ct	t ac	cgg	gcg	gt t	ct g	t t a	t ca	gcc	agt	t t g	at g	ggc	t t g	t t t	720
Lys	Ser	Tyr	Arg	Al a	Val	Leu	Leu	Ser	Al a	Ser	Leu	Met	G y	Leu	Phe	240
225					230				235					240		
at t	caa	cag	t gc	gga	t gg	t t g	t ct	cac	gat	t t t	ct a	cac	cat	cag	gt a	768
I le	G n	G n	Cys	G y	Trp	Leu	Ser	Hi s	Asp	Phe	Leu	Hi s	Hi s	G n	Val	
			245					250					255			
t t t	gag	aca	cgc	t gg	ct c	aat	gac	gt t	gt t	ggc	t at	gt g	gt c	ggc	aac	816
Phe	G u	Thr	Arg	Trp	Leu	Asn	Asp	Val	Val	G y	Tyr	Val	Val	G y	Asn	
		260					265						270			
gt t	gt t	ct g	gga	t t c	agt	gt c	t cg	t gg	t gg	aag	acc	aag	cac	aac	ct g	864
Val	Val	Leu	G y	Phe	Ser	Val	Ser	Tr p	Tr p	Lys	Thr	Lys	Hi s	Asn	Leu	
		275				280					285					
cat	cat	gct	gct	ccg	aat	gaa	t gc	gac	caa	aag	t ac	aca	ccg	at t	gat	912
Hi s	Hi s	Al a	Al a	Pro	Asn	G u	Cys	Asp	G n	Lys	Tyr	Thr	Pro	I le	Asp	
	290					295				300						
gag	gat	at t	gat	act	ct c	ccc	at c	at t	gct	t gg	agt	aaa	gat	ct c	t t g	960
G u	Asp	I le	Asp	Thr	Leu	Pro	I le	I le	Al a	Trp	Ser	Lys	Asp	Leu	Leu	320
305					310					315						
gcc	act	gt t	gag	agc	aag	acc	at g	t t g	cga	gt t	ct t	cag	t ac	cag	cac	1008
Al a	Thr	Val	G u	Ser	Lys	Thr	Met	Leu	Arg	Val	Leu	G n	Tyr	G n	Hi s	
			325					330					335			
ct a	t t c	t t t	t t g	gt t	ct t	t t g	acg	t t t	gcc	gcg	agt	t gg	ct a	t t t	t t t	1056
Leu	Phe	Phe	Leu	Val	Leu	Leu	Thr	Phe	Al a	Arg	Al a	Ser	Trp	Leu	Phe	
			340					345					350			
t gg	agc	gcg	gcc	t t c	act	ct c	agg	ccc	gag	t t g	acc	ct t	ggc	gag	aag	1104
Trp	Ser	Al a	Al a	Phe	Thr	Leu	Arg	Pro	G u	Leu	Thr	Leu	G y	G u	Lys	
		355					360						365			
ct t	t t g	gag	agg	gga	acg	at g	gct	t t g	cac	t ac	at t	t gg	t t t	aat	agt	1152
Leu	Leu	G u	Arg	G y	Thr	Met	Al a	Leu	Hi s	Tyr	I le	Trp	Phe	Asn	Ser	
		370				375					380					
gt t	gcg	t t t	t at	ct g	ct c	ccc	gga	t gg	aaa	cca	gt t	gt a	t gg	at g	gt g	1200
Val	Al a	Phe	Tyr	Leu	Leu	Pro	G y	Trp	Lys	Pro	Val	Val	Trp	Met	Val	400
385					390					395					400	
gt c	agc	gag	ct c	at g	t ct	ggt	t t c	ct g	ct g	gga	t ac	gt a	t t t	gt a	ct c	1248
Val	Ser	G u	Leu	Met	Ser	G y	Phe	Leu	Leu	G y	Tyr	Val	Phe	Val	Leu	
			405					410					415			
agt	cac	aat	gga	at g	gag	gt g	t ac	aat	acg	t ca	aag	gac	t t c	gt g	aat	1296
Ser	Hi s	Asn	G y	Met	G u	Val	Tyr	Asn	Thr	Ser	Lys	Asp	Phe	Val	Asn	
		420					425						430			
gcc	cag	at t	gca	t cg	act	cgc	gac	at c	aaa	gca	ggg	gt g	t t t	aat	gat	1344
Al a	G n	I le	Al a	Ser	Thr	Arg	Asp	I le	Lys	Al a	G y	Val	Phe	Asn	Asp	
		435					440					445				
t gg	t t c	acc	gga	ggt	ct c	aac	aga	cag	at t	gag	cat	cat	ct a	t t t	cca	1392
Trp	Phe	Thr	G y	G y	Leu	Asn	Arg	G n	I le	G u	Hi s	Hi s	Leu	Phe	Pro	

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450	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	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									

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 <212> PRT
 <213> Ceratodon purpureus

<400> 36

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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				

PF58307. txt

Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
 385 Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
 405 Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
 420 Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
 Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
 435 Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
 Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His Leu Phe Pro
 450 Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
 465 Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
 485 Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
 500 Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
 Ser His Gln Gln Leu Ala Ala Ser
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 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 atg 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 ctg 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 ctg 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 ctg 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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Tr p	Leu	Al a	Hi s	Asp	Phe	Leu	Hi s	G n	G n	Val	Phe	G u	Asn	Arg	Thr	
	210					215					220					
gcg	aac	t cc	t t c	t t t	ggc	t at	t t g	t t c	ggc	aat	t gc	gt g	ct t	ggc	t t t	720
Al a	Asn	Ser	Phe	Phe	G y	Tyr	Leu	Phe	G y	Asn	Cys	Val	Leu	G y	Phe	
225					230				235						240	
agt	gt a	t ca	t gg	t gg	agg	acg	aag	cac	aac	at t	cat	cat	act	gct	ccg	768
Ser	Val	Ser	Tr p	Tr p	Arg	Thr	Lys	Hi s	Asn	I le	Hi s	Hi s	Thr	Al a	Pro	
				245					250					255		
aat	gag	t gc	gac	gaa	cag	t ac	aca	cct	ct a	gac	gaa	gac	at t	gat	act	816
Asn	G u	Oys	Asp	G u	G n	Tyr	Thr	Pro	Leu	Asp	G u	Asp	I le	Asp	Thr	
			260					265					270			
ct c	ccc	at c	at t	gcc	t gg	agc	aag	gaa	at t	t t g	gcc	acc	gt t	gat	agc	864
Leu	Pro	I le	I le	Al a	Tr p	Ser	Lys	G u	I le	Leu	Al a	Thr	Val	G u	Ser	
		275					280					285				
aag	aga	at t	t t g	cga	gt g	ct t	caa	t at	cag	cac	t ac	at g	at t	ct g	cct	912
Lys	Arg	I le	Leu	Arg	Val	Leu	G n	Tyr	G n	Hi s	Tyr	Met	I le	Leu	Pro	
	290					295					300					
ct a	t t g	t t c	at g	gcc	cgg	t ac	agt	t gg	act	t t t	gga	agt	t t g	ct c	t t c	960
Leu	Leu	Phe	Met	Al a	Arg	Tyr	Ser	Tr p	Thr	Phe	G y	Ser	Leu	Leu	Phe	
305					310					315					320	
aca	t t c	aat	cct	gat	t t g	agc	acg	acc	aag	gga	t t g	at a	gag	aag	gga	1008
Thr	Phe	Asn	Pro	Asp	Leu	Ser	Thr	Thr	Lys	G y	Leu	I le	G u	Lys	G y	
				325					330					335		
aca	gt t	gct	t t t	cac	t ac	gcc	t gg	t t c	agt	t gg	gct	gcg	t t c	cat	at t	1056
Thr	Val	Al a	Phe	Hi s	Tyr	Al a	Tr p	Phe	Ser	Tr p	Al a	Al a	Phe	Hi s	I le	
			340					345					350			
t t g	ccg	ggt	gt c	gct	aag	cct	ct t	gcg	t gg	at g	gt a	gca	act	gat	ct t	1104
Leu	Pro	G y	Val	Al a	Lys	Pro	Leu	Al a	Tr p	Met	Val	Al a	Thr	G u	Leu	
		355					360					365				
gt g	gcc	ggt	t t g	t t g	t t g	gga	t t c	gt g	t t t	acg	t t g	agt	cac	aat	gga	1152
Val	Al a	G y	Leu	Leu	Leu	G y	Phe	Val	Phe	Thr	Leu	Ser	Hi s	Asn	G y	
	370					375					380					
aag	gag	gt t	t ac	aat	gaa	t cg	aag	gac	t t c	gt g	aga	gcc	cag	gt t	at t	1200
Lys	G u	Val	Tyr	Asn	G u	Ser	Lys	Asp	Phe	Val	Arg	Al a	G n	Val	I le	
385					390					395				400		
acc	acc	cg t	aac	acc	aag	cga	ggc	t gg	t t c	aac	gat	t gg	t t c	act	ggg	1248
Thr	Thr	Arg	Asn	Thr	Lys	Arg	G y	Tr p	Phe	Asn	Asp	Tr p	Phe	Thr	G y	
				405					410					415		
gga	ct c	gac	acc	cag	at t	gat	cat	cac	ct g	t t t	cca	aca	at g	ccc	agg	1296
G y	Leu	Asp	Thr	G n	I le	G u	Hi s	Hi s	Leu	Phe	Pro	Thr	Met	Pro	Arg	
			420					425					430			
cac	aac	t ac	ccc	aag	at c	gca	cct	cag	gt c	gag	gct	ct t	t gc	aag	aag	1344
Hi s	Asn	Tyr	Pro	Lys	I le	Al a	Pro	G n	Val	G u	Al a	Leu	Cys	Lys	Lys	
		435				440					445					
cac	ggc	ct c	gat	t ac	gat	aat	gt c	t cc	gt c	gt t	ggt	gcc	t ct	gt c	gcg	1392
Hi s	G y	Leu	G u	Tyr	Asp	Asn	Val	Ser	Val	Val	G y	Al a	Ser	Val	Al a	
	450				455					460						
gt t	gt g	aag	gcg	ct c	aag	gaa	at t	gct	gat	gaa	gcg	t ca	at t	cg g	ct t	1440
Val	Val	Lys	Al a	Leu	Lys	G u	I le	Al a	Asp	G u	Al a	Ser	I le	Arg	Leu	
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cac	gct	cac	t aa													1452
Hi s	Al a	Hi s														

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 <211> 483
 <212> PRT
 <213> Cer at odon pur pur eus

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 20 25 30
 Leu Lys Hi s Al a Lys Lys Val Ser Al a G n G y Lys Thr Al a G y G n
 35 40 45
 Thr Leu Arg G n Arg Ser Val G n Asp Lys Lys Pro G y Thr Tyr Ser
 Sei t e 46

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50 55 60
 Leu Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp Met Ile
 65 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 Glu 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 Glu Thr 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 Glu Glu Cys Gly
 195 200 205
 Trp Leu Ala His Asp Phe Leu His Glu Glu 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 Glu 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 Glu Tyr Glu 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 Glu 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 Glu Ile Glu His His Leu Phe Pro Thr Met Pro Arg
 420 425 430
 His Asn Tyr Pro Lys Ile Ala Pro Glu 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
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 Val Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile Arg Leu
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 His Ala His

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 <213> Mbr tierella alpina

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 Sei te 47

PF58307. txt

1	aat	gcc	gag	gct	ct g	aat	gag	ggc	aag	aag	gat	gcc	gag	gca	ccc	t t c	96
	Asn	Al a	Gl u	Al a	Leu	Asn	Gl u	Gl y	Lys	Lys	Asp	Al a	Gl u	Al a	Pro	Phe	
				20					25					30			
	t t g	at g	at c	at c	gac	aac	aag	gt g	t ac	gat	gt c	cgc	gag	t t c	gt c	cct	144
	Leu	Met	l l e	l l e	Asp	Asn	Lys	Val	Tyr	Asp	Val	Arg	Gl u	Phe	Val	Pro	
				35				40					45				
	gat	cat	ccc	ggt	gga	agt	gt g	at t	ct c	acg	cac	gt t	ggc	aag	gac	ggc	192
	Asp	His	Pro	Gl y	Gl y	Ser	Val	l l e	Leu	Thr	His	Val	Gl y	Lys	Asp	Gl y	
		50					55					60					
	act	gac	gt c	t t t	gac	act	t t t	cac	ccc	gag	gct	gct	t gg	gag	act	ct t	240
	Thr	Asp	Val	Phe	Asp	Thr	Phe	His	Pro	Gl u	Al a	Al a	Tr p	Gl u	Thr	Leu	
	65					70				75					80		
	gcc	aac	t t t	t ac	gt t	ggt	gat	at t	gac	gag	agc	gac	cgc	gat	at c	aag	288
	Al a	Asn	Phe	Tyr	Val	Gl y	Asp	l l e	Asp	Gl u	Ser	Asp	Arg	Asp	l l e	Lys	
					85					90					95		
	aat	gat	gac	t t t	gcg	gcc	gag	gt c	cgc	aag	ct g	cgt	acc	t t g	t t c	cag	336
	Asn	Asp	Asp	Phe	Al a	Al a	Gl u	Val	Arg	Lys	Leu	Arg	Thr	Leu	Phe	Gl n	
				100					105					110			
	t ct	ct t	ggt	t ac	t ac	gat	t ct	t cc	aag	gca	t ac	t ac	gcc	t t c	aag	gt c	384
	Ser	Leu	Gl y	Tyr	Tyr	Asp	Ser	Ser	Lys	Al a	Tyr	Tyr	Al a	Phe	Lys	Val	
			115				120						125				
	t cg	t t c	aac	ct c	t gc	at c	t gg	ggt	t t g	t cg	acg	gt c	at t	gt g	gcc	aag	432
	Ser	Phe	Asn	Leu	Cys	l l e	Tr p	Gl y	Leu	Ser	Thr	Val	l l e	Val	Al a	Lys	
		130					135					140					
	t gg	ggc	cag	acc	t cg	acc	ct c	gcc	aac	gt g	ct c	t cg	gct	gcg	ct t	t t g	480
	Tr p	Gl y	Gl n	Thr	Ser	Thr	Leu	Al a	Asn	Val	Leu	Ser	Al a	Al a	Leu	Leu	
	145				150					155					160		
	ggt	ct g	t t c	t gg	cag	cag	t gc	gga	t gg	t t g	gct	cac	gac	t t t	t t g	cat	528
	Gl y	Leu	Phe	Tr p	Gl n	Gl n	Cys	Gl y	Tr p	Leu	Al a	His	Asp	Phe	Leu	His	
					165				170						175		
	cac	cag	gt c	t t c	cag	gac	cgt	t t c	t gg	ggt	gat	ct t	t t c	ggc	gcc	t t c	576
	His	Gl n	Val	Phe	Gl n	Asp	Arg	Phe	Tr p	Gl y	Asp	Leu	Phe	Gl y	Al a	Phe	
				180					185					190			
	t t g	gga	ggt	gt c	t gc	cag	ggc	t t c	t cg	t cc	t cg	t gg	t gg	aag	gac	aag	624
	Leu	Gl y	Gl y	Val	Cys	Gl n	Gl y	Phe	Ser	Ser	Ser	Tr p	Tr p	Lys	Asp	Lys	
			195					200						205			
	cac	aac	act	cac	cac	gcc	gcc	ccc	aac	gt c	cac	ggc	gag	gat	ccc	gac	672
	His	Asn	Thr	His	His	Al a	Al a	Pro	Asn	Val	His	Gl y	Gl u	Asp	Pro	Asp	
						215						220					
	at t	gac	acc	cac	cct	ct g	t t g	acc	t gg	agt	gag	cat	gcg	t t g	gag	at g	720
	l l e	Asp	Thr	His	Pro	Leu	Leu	Thr	Tr p	Ser	Gl u	His	Al a	Leu	Gl u	Met	
	225					230					235					240	
	t t c	t cg	gat	gt c	cca	gat	gag	gag	ct g	acc	cgc	at g	t gg	t cg	cgt	t t c	768
	Phe	Ser	Asp	Val	Pro	Asp	Gl u	Gl u	Leu	Thr	Arg	Met	Tr p	Ser	Arg	Phe	
					245				250						255		
	at g	gt c	ct g	aac	cag	acc	t gg	t t t	t ac	t t c	ccc	at t	ct c	t cg	t t t	gcc	816
	Met	Val	Leu	Asn	Gl n	Thr	Tr p	Phe	Tyr	Phe	Pro	l l e	Leu	Ser	Phe	Al a	
				260				265						270			
	cgt	ct c	t cc	t gg	t gc	ct c	cag	t cc	at t	ct c	t t t	gt g	ct g	cct	aac	ggt	864
	Arg	Leu	Ser	Tr p	Cys	Leu	Gl n	Ser	l l e	Leu	Phe	Val	Leu	Pro	Asn	Gl y	
			275					280					285				
	cag	gcc	cac	aag	ccc	t cg	ggc	gcg	cgt	gt g	ccc	at c	t cg	t t g	gt c	gag	912
	Gl n	Al a	His	Lys	Pro	Ser	Gl y	Al a	Arg	Val	Pro	l l e	Ser	Leu	Val	Gl u	
						295						300					
	cag	ct g	t cg	ct t	gcg	at g	cac	t gg	acc	t gg	t ac	ct c	gcc	acc	at g	t t c	960
	Gl n	Leu	Ser	Leu	Al a	Met	His	Tr p	Thr	Tr p	Tyr	Leu	Al a	Thr	Met	Phe	
	305				310					315						320	
	ct g	t t c	at c	aag	gat	ccc	gt c	aac	at g	ct g	gt g	t ac	t t t	t t g	gt g	t cg	1008
	Leu	Phe	l l e	Lys	Asp	Pro	Val	Asn	Met	Leu	Val	Tyr	Phe	Leu	Val	Ser	
					325				330						335		
	cag	gcg	gt g	t gc	gga	aac	t t g	t t g	gcg	at c	gt g	t t c	t cg	ct c	aac	cac	1056
	Gl n	Al a	Val	Cys	Gl y	Asn	Leu	Leu	Al a	l l e	Val	Phe	Ser	Leu	Asn	His	
				340					345					350			
	aac	ggt	at g	cct	gt g	at c	t cg	aag	gag	gag	gcg	gt c	gat	at g	gat	t t c	1104
	Asn	Gl y	Met	Pro	Val	l l e	Ser	Lys	Gl u	Gl u	Al a	Val	Asp	Met	Asp	Phe	
			355					360					365				
	t t c	acg	aag	cag	at c	at c	acg	ggt	cgt	gat	gt c	cac	ccg	ggt	ct a	t t t	1152

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Phe	Thr	Lys	G n	I l e	I l e	Thr	G y	Arg	Asp	Val	Hi s	Pro	G y	Leu	Phe		
	370					375					380						
gcc	aac	tgg	t t c	acg	ggt	gga	t t g	aac	t at	cag	at c	gag	cac	cac	t t g		1200
Al a	Asn	Tr p	Phe	Thr	G y	G y	Leu	Asn	Tyr	G n	I l e	G u	Hi s	Hi s	Leu		
	385				390					395					400		
t t c	cct	t cg	at g	cct	cgc	cac	aac	t t t	t ca	aag	at c	cag	cct	gct	gt c		1248
Phe	Pro	Ser	M et	Pro	Arg	Hi s	Asn	Phe	Ser	Lys	I l e	G n	Pro	Al a	Val		
				405				410						415			
gag	acc	ct g	t gc	aaa	aag	t ac	aat	gt c	cga	t ac	cac	acc	acc	gg t	at g		1296
G u	Thr	Leu	Cys	Lys	Lys	Tyr	Asn	Val	Arg	Tyr	Hi s	Thr	Thr	G y	M et		
			420					425					430				
at c	gag	gga	act	gca	gag	gt c	t t t	agc	cgt	ct g	aac	gag	gt c	t cc	aag		1344
I l e	G u	G y	Thr	Al a	G u	Val	Phe	Ser	Arg	Leu	Asn	G u	Val	Ser	Lys		
		435				440						445					
gct	gcc	t cc	aag	at g	ggt	aag	gcg	cag	t aa								1374
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<212> PRT

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			20					25					30				
Leu	M et	I l e	I l e	Asp	Asn	Lys	Val	Tyr	Asp	Val	Arg	G u	Phe	Val	Pro		
		35				40						45					
Asp	Hi s	Pro	G y	G y	Ser	Val	I l e	Leu	Thr	Hi s	Val	G y	Lys	Asp	G y		
	50					55					60						
Thr	Asp	Val	Phe	Asp	Thr	Phe	Hi s	Pro	G u	Al a	Al a	Tr p	G u	Thr	Leu		
65					70					75				80			
Al a	Asn	Phe	Tyr	Val	G y	Asp	I l e	Asp	G u	Ser	Asp	Arg	Asp	I l e	Lys		
				85					90					95			
Asn	Asp	Asp	Phe	Al a	Al a	G u	Val	Arg	Lys	Leu	Arg	Thr	Leu	Phe	G n		
			100					105					110				
Ser	Leu	G y	Tyr	Tyr	Asp	Ser	Ser	Lys	Al a	Tyr	Tyr	Al a	Phe	Lys	Val		
		115				120						125					
Ser	Phe	Asn	Leu	Cys	I l e	Tr p	G y	Leu	Ser	Thr	Val	I l e	Val	Al a	Lys		
	130					135					140						
Tr p	G y	G n	Thr	Ser	Thr	Leu	Al a	Asn	Val	Leu	Ser	Al a	Al a	Leu	Leu		
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G y	Leu	Phe	Tr p	G n	Cys	G y	Tr p	Leu	Al a	Hi s	Asp	Phe	Leu	Hi s			
				165				170					175				
Hi s	G n	Val	Phe	G n	Asp	Arg	Phe	Tr p	G y	Asp	Leu	Phe	G y	Al a	Phe		
			180					185					190				
Leu	G y	G y	Val	Cys	G n	G y	Phe	Ser	Ser	Ser	Tr p	Tr p	Lys	Asp	Lys		
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Hi s	Asn	Thr	Hi s	Hi s	Al a	Al a	Pro	Asn	Val	Hi s	G y	G u	Asp	Pro	Asp		
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I l e	Asp	Thr	Hi s	Pro	Leu	Leu	Thr	Tr p	Ser	G u	Hi s	Al a	Leu	G u	M et		
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Phe	Ser	Asp	Val	Pro	Asp	G u	G u	Leu	Thr	Arg	M et	Tr p	Ser	Arg	Phe		
				245					250					255			
M et	Val	Leu	Asn	G n	Thr	Tr p	Phe	Tyr	Phe	Pro	I l e	Leu	Ser	Phe	Al a		
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Arg	Leu	Ser	Tr p	Cys	Leu	G n	Ser	I l e	Leu	Phe	Val	Leu	Pro	Asn	G y		
		275					280						285				
G n	Al a	Hi s	Lys	Pro	Ser	G y	Al a	Arg	Val	Pro	I l e	Ser	Leu	Val	G u		
						295						300					
G n	Leu	Ser	Leu	Al a	M et	Hi s	Tr p	Thr	Tr p	Tyr	Leu	Al a	Thr	M et	Phe		
305					310					315							
Leu	Phe	I l e	Lys	Asp	Pro	Val	Asn	M et	Leu	Val	Tyr	Phe	Leu	Val	Ser		
				325					330					335			
G n	Al a	Val	Cys	G y	Asn	Leu	Leu	Al a	I l e	Val	Phe	Ser	Leu	Asn	Hi s		
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Asn Gly Met Pro Val Ile Ser Lys Gu Gu Ala Val Asp Met Asp Phe
 355 360 365
 Phe Thr Lys Gn Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
 370 375 380
 Ala Asn Trp Phe Thr Gly Leu Asn Tyr Gn Ile Gu His His Leu
 385 390 395 400
 Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gn Pro Ala Val
 405 410 415
 Gu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
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 Ile Gu Gly Thr Ala Gu Val Phe Ser Arg Leu Asn Gu Val Ser Lys
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 Ala Ala Ser Lys Met Gly Lys Ala Gn
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 Asn Ala Gu Ala Leu Asn Gu Gy Lys Lys Asp Ala Gu 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 Gu Phe Val Pro
 35 40 45
 gat cat ccc ggt gga agt gtg att ctg acg cac gtt ggc aag gac ggc 192
 Asp His Pro Gy Gy Ser Val Ile Leu Thr His Val Gy Lys Asp Gy
 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 Gu Ala Ala Trp Gu 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 Gy Asp Ile Asp Gu 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 Gu Val Arg Lys Leu Arg Thr Leu Phe Gn
 100 105 110
 tcc ctt ggc tac tac gac tgc tcc aag gca tac tat gcc ttc aag gtc 384
 Ser Leu Gy Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
 115 120 125
 tgc ttc aac ctg tgc atc tgg ggc ttg tgc act ttc att gtt gcc aag 432
 Ser Phe Asn Leu Cys Ile Trp Gy Leu Ser Thr Phe Ile Val Ala Lys
 130 135 140
 tgg ggc cag acc tgc acc ctg gcc aac gtg ctg tgc gct gcg ctg ttg 480
 Trp Gy Gn 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 gcg cac gac ttt ttg cac 528
 Gy Leu Phe Trp Gn Gn Cys Gy 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 Gn Val Phe Gn Asp Arg Phe Trp Gy Asp Leu Phe Gy Ala Phe
 180 185 190
 ttg gga ggt gtc tgc cag ggt ttc tgc tcc tcc tgg tgg aag gac aag 624
 Leu Gy Gy Val Cys Gn Gy 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 Gy Gu 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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I l e A s p T h r H i s P r o L e u L e u T h r T r p S e r G l u H i s A l a L e u G l u M e t
 225 230 235 240
 t t c t c g g a t g t t c c t g a c g a g g a g c t g a c c c g t a t g t g g t c g c g c t t c
 P h e S e r A s p V a l P r o A s p G l u G l u L e u T h r A r g M e t T r p S e r A r g P h e 768
 245 250 255
 a t g g t c c t c a a c c a g a c c t g g t t c t a c t t c c c c a t t c t c t c g t t t g c c
 M e t V a l L e u A s n G l n T h r T r p P h e T y r P h e P r o I l e L e u S e r P h e A l a 816
 260 265 270
 c g t c t g t c c t g g t g c c t c c a g t c c a t t a t g t t t g t t c t g c c c a a c g g t
 A r g L e u S e r T r p C y s L e u G l n S e r I l e M e t P h e V a l L e u P r o A s n G l y 864
 275 280
 c a g g c c a c a a g c c c t c t g g a g c g c g t g t g c c c a t t t c g t t g g t c g a g
 G l n A l a H i s L y s P r o S e r G l y A l a A r g V a l P r o I l e S e r L e u V a l G l u 912
 290 295 300
 c a g c t g t c t c t g g c t a t g c a c t g g a c c t g g t a c c t c g c c a c c a t g t t c
 G l n L e u S e r L e u A l a M e t H i s T r p T h r T r p T y r L e u A l a T h r M e t P h e 960
 305 310 315 320
 c t g t t c a t t a a g g a t c c c g t c a a c a t g a t t g t g t a c t t t t t g g t g t c g
 L e u P h e I l e L y s A s p P r o V a l A s n M e t I l e V a l T y r P h e L e u 1008
 325 330 335
 c a g g c t g t t t g c g g c a a c t t g t t g g c g a t t g t g t t c t c g c t c a a c c a c
 G l n A l a V a l C y s G l y A s n L e u L e u A l a I l e V a l P h e S e r L e u A s n H i s 1056
 340 345 350
 a a c g g c a t g c c t g t g a t c t c c a a g g a g g a a g c g g t c g a t a t g g a c t t c
 A s n G l y M e t P r o V a l I l e S e r L y s G l u G l u A l a V a l A s p M e t A s p P h e 1104
 355 360 365
 t t c a c c a a g c a g a t c a t c a c g g g t c g t g a t g t t c a c c c t g g t c t g t t t
 P h e T h r L y s G l n I l e I l e T h r G l y A r g A s p V a l H i s P r o G l y L e u P h e 1152
 370 375 380
 g c c a a c t g g t t c a c g g g t g g a t t g a a c t a c c a g a t t g a g c a c c a c t t g
 A l a A s n T r p P h e T h r G l y G l y L e u A s n T y r G l n I l e G l u H i s H i s L e u 1200
 385 390 395 400
 t t c c c t t c g a t g c c c c g c c a c a a c t t t t c a a a g a t c c a g c c t g c t g t c
 P h e P r o S e r M e t P r o A r g H i s A s n P h e S e r L y s I l e G l n P r o A l a V a l 1248
 405 410 415
 g a g a c t t t g t g c a a a a a g t a c g g t g t c c g a t a c c a t a c c a c t g g t a t g
 G l u T h r L e u C y s L y s L y s T y r G l y V a l A r g T y r H i s T h r 1296
 420 425 430 435
 a t c g a g g g a a c t g c a g a g g t c t t t a g c c g t t t g a a c g a g g t c t c c a a g
 I l e G l u G l y T h r A l a G l u V a l P h e S e r A r g L e u A s n G l u V a l S e r L y s 1344
 440 445
 g c g g c c t c c a a g a t g g g c a a g g c a c a g t a a
 A l a A l a S e r L y s M e t G l y L y s A l a G l n 1374
 450 455

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 1 5 10 15
 A s n A l a G l u A l a L e u A s n G l u G l y L y s L y s A s p A l a G l u A l a P r o P h e
 20 25 30
 L e u M e t I l e I l e A s p A s n L y s V a l T y r A s p V a l A r g G l u P h e V a l P r o
 35 40 45
 A s p H i s P r o G l y G l y S e r V a l I l e L e u T h r H i s V a l G l y L y s A s p G l y
 50 55 60
 T h r A s p V a l P h e A s p T h r P h e H i s P r o G l u A l a T r p G l u T h r L e u
 65 70 75 80
 A l a A s n P h e T y r V a l G l y A s p I l e A s p G l u S e r A s p A r g A l a I l e L y s
 85 90 95
 A s n A s p A s p P h e A l a A l a G l u V a l A r g L y s L e u A r g T h r L e u P h e G l n
 100 105 110
 S e r L e u G l y T y r T y r A s p S e r S e r L y s A l a T y r T y r A l a P h e L y s V a l
 115 120 125
 S e r P h e A s n L e u C y s I l e T r p G l y L e u S e r T h r P h e I l e V a l A l a L y s
 S e i t e 51

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130 135 140
 Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
 145 Gly Leu Phe Trp Gln 150 Cys Gly Trp Leu 155 His Asp Phe Leu 160
 His Gln Val Phe 165 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
 His Asn Thr His His Ala Ala Pro Asn Val His Gly Gu Asp Pro Asp
 210 215 220
 Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Gu Met
 225 230 235 240
 Phe Ser Asp Val Pro Asp Gu Gu 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 Gu
 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 Gu Gu 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 Gu 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
 Gu Thr Leu Cys Lys Lys Tyr Gly Val Arg Tyr His Thr Thr Gly Met
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 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 Gn Gn
 130 135 140
 xaa Gly Trp Leu Ala His Asp Phe xaa His His Gn 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
 Gn 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 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 Gly 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 xaa xaa xaa 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 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 Gn
 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 Gn Ile Gu His His
 385 390 395 400

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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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- <223> xaa in position 9 is Leu or Val
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 xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Leu Cys xaa Lys
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<222> (27)..(27)

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 Asn xaa His His xaa xaa xaa Asn xaa xaa xaa
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<223> xaa in position 26 is any or no amino acid

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 xaa xaa xaa xaa Asn xaa xaa Gly Met xaa
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<400> 48
 Pro Asp Ile Asp Thr xaa Pro xaa Leu Leu xaa Trp xaa
 1 5 10

<210> 49
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 1 5 10 15
 Trp

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 1 5 10 15
 xaa xaa xaa xaa
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<220>
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Gu Ala Asn Gly Asp Lys Arg Lys Thr lle Leu lle Gu Gly Val Leu
20
t ac gac gcg acg aac t t t aag cac cgc ggc ggt t cg at c at c aac t t c      144
Tyr Asp Ala Thr Asn Phe Lys His Pro Gly Gly Ser lle lle Asn Phe
35
t t g acc gag ggc gag gcc ggc gt g gac gcg acg cag gcg t ac cgc gag      192
Leu Thr Gu Gly Gu Ala Gly Val Asp Ala Thr Gn Ala Tyr Arg Gu
50
t t t cat cag cgg t cc ggc aag gcc gac aag t ac ct c aag t cg ct g ccg      240
Phe His Gn Arg Ser Gly Lys Ala Asp Lys Tyr Leu Lys Ser Leu Pro
65
aag ct g gat gcg t cc aag gt g gag t cg cgg t t c t cg gcc aaa gag cag      288
Lys Leu Asp Ala Ser Lys Val Gu Ser Arg Phe Ser Ala Lys Gu Gn
85
gcg cgg cgc gac gcc at g acg cgc gac t ac gcg gcc t t t cgc gag gag      336
Ala Arg Arg Asp Ala Met Thr Arg Asp Tyr Ala Ala Phe Arg Gu Gu
100
ct c gt c gcc gag ggg t ac t t t gac ccg t cg at c ccg cac at g at t t ac      384
Leu Val Ala Gu Gly Tyr Phe Asp Pro Ser lle Pro His Met lle Tyr
115
cgc gt c gt g gag at c gt g gcg ct c t t c gcg ct c t cg t t c t gg ct c at g      432
Arg Val Val Gu lle Val Ala Leu Phe Ala Leu Ser Phe Trp Leu Met
130
t cc aag gcc t cg ccc acc t cg ct c gt g ct g ggc gt g gt g at g aac ggc      480
Ser Lys Ala Ser Pro Thr Ser Leu Val Leu Gly Val Val Met Asn Gly
145
at t gcg cag ggc cgc t gc ggc t gg gt c at g cac gag at g ggc cac ggc      528
lle Ala Gn Gly Arg Cys Gly Trp Val Met His Gu Met Gly His Gly
165
t cg t t c acg ggc gt c at c t gg ct c gac gac cgg at g t gc gag t t c t t c      576
Ser Phe Thr Gly Val lle Trp Leu Asp Asp Arg Met Cys Gu Phe Phe
180
t ac ggc gt c ggc t gc ggc at g agc ggg cac t ac t gg aag aac cag cac      624
Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gn His
195
agc aag cac cac gcc gcg ccc aac cgc ct c gag cac gat ct c      672
Ser Lys His His Ala Ala Pro Asn Arg Leu Gu His Asp Val Asp Leu
210
aac acg ct g ccc ct g gt c gcc t t t aac gag cgc gt c gt g cgc aag gt c      720
Asn Thr Leu Pro Leu Val Ala Phe Asn Gu Arg Val Val Arg Lys Val
225
aag ccg gga t cg ct g ct g gcg ct c t gg ct g cgc gt g cag gcg t ac ct c      768
Lys Pro Gly Ser Leu Leu Ala Leu Trp Leu Arg Val Gn Ala Tyr Leu
245
t t t gcg ccc gt c t cg t gc ct g ct c at c ggc ct t ggc t gg acg ct c t ac      816
Phe Ala Pro Val Ser Cys Leu Leu lle Gly Leu Gly Trp Thr Leu Tyr
260
ct g cac ccg cgc t ac at g ct g cgc acc aag cgg cac at g gag t t c gt c      864
Leu His Pro Arg Tyr Met Leu Arg Thr Lys Arg His Met Gu Phe Val
275
t gg at c t t c gcg cgc t ac at t ggc t gg t t c t cg ct c at g ggc gct ct c      912
Trp lle Phe Ala Arg Tyr lle Gly Trp Phe Ser Leu Met Gly Ala Leu
290
ggc t ac t cg ccg ggc acc t cg Ser Val Gly Met Tyr Leu Cys Ser Phe Gly      960
Gly Tyr Ser Pro Gly Thr Ser Val Gly Met Tyr Leu Cys Ser Phe Gly
305
ct c ggc t gc at t t ac at t t t c ct g cag t t c gcc gt c agc cac acg cac      1008
Leu Gly Cys lle Tyr lle Phe Leu Gn Phe Ala Val Ser His Thr His
325
ct g ccg gt g acc aac ccg gag gac cag ct g cac t gg ct c gag t ac gcg      1056
Leu Pro Val Thr Asn Pro Gu Asp Gn Leu His Trp Leu Gu Tyr Ala
340
345
350

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gcc gac cac acg gt g aac att agc acc aag t cc t gg ct c gt c acg t gg 1104
Al a Asp Hi s Thr Val Asn lle Ser Thr Lys Ser Trp Leu Val Thr Trp
355 360 365
t gg at g t cg aac ct g aac t t t cag at c gag cac cac ct c t t c ccc acg 1152
Tr p Met Ser Asn Leu Asn Phe G n lle G u Hi s Hi s Leu Phe Pro Thr
370 375 380
gcg ccg cag t t c cgc t t c aag gaa at c agt cct cgc gt c gag gcc ct c 1200
Al a Pro G n Phe Arg Phe Lys G u lle Ser Pro Arg Val G u Al a Leu
385 390 395 400
t t c aag cgc cac aac ct c ccg t ac t ac gac ct g ccc t ac acg agc gcg 1248
Phe Lys Arg Hi s Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Al a
405 410 415
gt c t cg acc acc t t t gcc aat ct t t at t cc gt c ggc cac t cg gt c ggc 1296
Val Ser Thr Thr Phe Al a Asn Leu Tyr Ser Val G y Hi s Ser Val G y
420 425 430
gcc gac acc aag aag cag gac t ga 1320
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435

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<211> 439

<212> PRT

<213> Thr aust ochyt r i um ssp.

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Tyr Asp Al a Thr Asn Phe Lys Hi s Pro G y G y Ser lle lle Asn Phe
35 40 45
Leu Thr G u G y G u Al a G y Val Asp Al a Thr G n Al a Tyr Arg G u
50 55 60
Phe Hi s G n Arg Ser G y Lys Al a Asp Lys Tyr Leu Lys Ser Leu Pro
65 70 75 80
Lys Leu Asp Al a Ser Lys Val G u Ser Arg Phe Ser Al a Lys G u G n
85 90 95
Al a Arg Arg Asp Al a Met Thr Arg Asp Tyr Al a Al a Phe Arg G u G u
100 105 110
Leu Val Al a G u G y Tyr Phe Asp Pro Ser lle Pro Hi s Met lle Tyr
115 120 125
Arg Val Val G u lle Val Al a Leu Phe Al a Leu Ser Phe Tr p Leu Met
130 135 140
Ser Lys Al a Ser Pro Thr Ser Leu Val Leu G y Val Val Met Asn G y
145 150 155 160
lle Al a G n G y Arg Cys G y Tr p Val Met Hi s G u Met G y Hi s G y
165 170 175 180
Ser Phe Thr G y Val lle Tr p Leu Asp Asp Arg Met Cys G u Phe Phe
180 185 190
Tyr G y Val G y Cys G y Met Ser G y Hi s Tyr Tr p Lys Asn G n Hi s
195 200 205
Ser Lys Hi s Hi s Al a Al a Pro Asn Arg Leu G u Hi s Asp Val Asp Leu
210 215 220
Asn Thr Leu Pro Leu Val Al a Phe Asn G u Arg Val Val Arg Lys Val
225 230 235 240
Lys Pro G y Ser Leu Leu Al a Leu Tr p Leu Arg Val G n Al a Tyr Leu
245 250 255
Phe Al a Pro Val Ser Cys Leu Leu lle G y Leu G y Tr p Thr Leu Tyr
260 265 270
Leu Hi s Pro Arg Tyr Met Leu Arg Thr Lys Arg Hi s Met G u Phe Val
275 280 285
Tr p lle Phe Al a Arg Tyr lle G y Tr p Phe Ser Leu Met G y Al a Leu
290 295 300
G y Tyr Ser Pro G y Thr Ser Val G y Met Tyr Leu Cys Ser Phe G y
305 310 315 320
Leu G y Cys lle Tyr lle Phe Leu G n Phe Al a Val Ser Hi s Thr Hi s
325 330 335
Leu Pro Val Thr Asn Pro G u Asp G n Leu Hi s Tr p Leu G u Tyr Al a
Sei te 65

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Al a Asp Hi s Thr Val Asn Ile Ser Thr Lys Ser Trp Leu Val Thr Trp
 340 345 350
 355
 Tr p Met Ser Asn Leu Asn Phe Gn Ile Gu Hi s Hi s Leu Phe Pro Thr
 370 375 380
 Al a Pro Gn Phe Arg Phe Lys Gu Ile Ser Pro Arg Val Gu Al a Leu
 385 390 395 400
 Phe Lys Arg Hi s Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Al a
 405 410 415
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 420 425 430
 Al a Asp Thr Lys Lys Gn Asp
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 <213> *Ostreococcus tauri*

<220>
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 <222> (1).. (1371)

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 gcg ttc gac ggt gag cgc gag cgg gcg gag gca aac gt g aag ct g tcc 96
 Al a Phe Asp Gy Gu Arg Gu Arg Al a Gu Al a Asn Val Lys Leu Ser
 20 25 30
 gcg gag aag at g gag ccg gcg gcg ct g gcg aag acg ttc gcg agg cgg 144
 Al a Gu Lys Met Gu Pro Al a Al a Leu Al a Lys Thr Phe Al a Arg Arg
 35 40 45
 t ac gt c gt g at c gag ggg gt g gag t ac gat gt g acg gat ttt aag cac 192
 Tyr Val Val Ile Gu Gy Val Gu Tyr Asp Val Thr Asp Phe Lys Hi s
 50 55 60
 ccg gga gga acg gtt att ttc tat gcg ttg tca aac acc ggg gcg gac 240
 Pro Gy Gy Thr Val Ile Phe Tyr Al a Leu Ser Asn Thr Gy Al a Asp
 65 70 75 80
 gcg acg gaa gcg ttc aag gag ttt cat cat cgg t cg aga aag gcg agg 288
 Al a Thr Gu Al a Phe Lys Gu Phe Hi s Hi s Arg Ser Arg Lys Al a Arg
 85 90 95
 aaa gcc ttg gcg gcg ct c ccg tct cga ccg gcc aag acg gcc aag gt g 336
 Lys Al a Leu Al a Al a Leu Pro Ser Arg Pro Al a Lys Thr Al a Lys Val
 100 105 110
 gac gac gcg gag at g ct c caa gat ttc gcc aag tgg cgg aaa gaa ttg 384
 Asp Asp Al a Gu Met Leu Gn Asp Phe Al a Lys Trp Arg Lys Gu Leu
 115 120 125
 gag aga gat gga ttc ttc aag ccc tct ccg gcg cac gt g gcg tat cgc 432
 Gu Arg Asp Gy Phe Phe Lys Pro Ser Pro Al a Hi s Val Al a Tyr Arg
 130 135 140
 ttc gcc gag ct c gcg gcg at g tac gct ct c ggg acg tac ct g at g tac 480
 Phe Al a Gu Leu Al a Al a Met Tyr Al a Leu Gy Thr Tyr Leu Met Tyr
 145 150 155 160
 gct cga tac gt c gt c tcc t cg gt g ct c gt g tac gct tgc ttt ttc ggc 528
 Al a Arg Tyr Val Val Ser Ser Val Leu Val Tyr Al a Cys Phe Phe Gy
 165 170 175
 gcc cga tgc ggt tgg gt g cag cac gag ggc gga cac agc t cg ct g acg 576
 Al a Arg Cys Gy Trp Val Gn Hi s Gu Gy Gy Hi s Ser Ser Leu Thr
 180 185 190
 ggc aac att tgg tgg gac aag cgc at c cag gcc ttc aca gcc ggg ttc 624
 Gy Asn Ile Trp Trp Asp Lys Arg Ile Gn Al a Phe Thr Al a Gy Phe
 195 200 205
 ggt ct c gcc ggt agc ggc gac at g tgg aac t cg at g cac aag cat 672
 Gy Leu Al a Gy Ser Gy Asp Met Trp Asn Ser Met Hi s Asn Lys Hi s
 210 215 220
 cac gcg acg cct caa aag gtt cgt cac gac at g gat ct g gac acc acc 720
 Hi s Al a Thr Pro Gn Lys Val Arg Hi s Asp Met Asp Leu Asp Thr Thr

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225	ccc	gcg	gt g	gcg	t t c	t t c	aac	acc	gcg	gt g	gaa	gac	aat	cgt	ccc	cgt	768
	Pro	Al a	Val	Al a	Phe	Phe	Asn	Thr	Al a	Val	Gl u	Asp	Asn	Arg	Pro	Arg	
					245					250					255		
	ggc	t t t	agc	aag	t ac	t gg	t t g	cgc	ct t	cag	gcg	t gg	acc	t t c	at c	ccc	816
	Gl y	Phe	Ser	Lys	Tyr	Trp	Leu	Arg	Leu	Gl n	Al a	Trp	Thr	Phe	I l e	Pro	
				260					265					270			
	gt g	acg	t cc	ggc	t t g	gt g	ct c	ct t	t t c	t gg	at g	t t t	t t c	ct c	cac	ccc	864
	Val	Thr	Ser	Gl y	Leu	Val	Leu	Leu	Phe	Trp	Met	Phe	Phe	Leu	Hi s	Pro	
				275					280				285				
	t cc	aag	gct	t t g	aag	ggc	ggc	aag	t ac	gaa	gag	t t g	gt g	t gg	at g	ct c	912
	Ser	Lys	Al a	Leu	Lys	Gl y	Gl y	Lys	Tyr	Gl u	Gl u	Leu	Val	Trp	Met	Leu	
				290			295					300					
	gcc	gcg	cac	gt c	at c	cgc	acg	t gg	acg	at c	aag	gcg	gt g	acc	gga	t t c	960
	Al a	Al a	Hi s	Val	I l e	Arg	Thr	Trp	Thr	I l e	Lys	Al a	Val	Thr	Gl y	Phe	
	305					310					315					320	
	acc	gcg	at g	cag	t cc	t ac	ggc	t t a	t t t	t t g	gcg	acg	agc	t gg	gt g	agc	1008
	Thr	Al a	Met	Gl n	Ser	Tyr	Gl y	Leu	Phe	Leu	Al a	Thr	Ser	Trp	Val	Ser	
					325					330					335		
	ggc	t gc	t at	ct g	t t t	gca	cac	t t c	t cc	acg	t cg	cac	acg	cac	ct g	gat	1056
	Gl y	Cys	Tyr	Leu	Phe	Al a	Hi s	Phe	Ser	Thr	Ser	Hi s	Thr	Hi s	Leu	Asp	
				340				345					350				
	gt g	gt g	ccc	gcg	gac	gag	cat	ct c	t cc	t gg	gt t	cga	t ac	gcc	gt c	gat	1104
	Val	Val	Pro	Al a	Asp	Gl u	Hi s	Leu	Ser	Trp	Val	Arg	Tyr	Al a	Val	Asp	
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	cac	acg	at c	gac	at c	gat	ccg	agt	caa	ggc	t gg	gt g	aac	t gg	t t g	at g	1152
	Hi s	Thr	I l e	Asp	I l e	Asp	Pro	Ser	Gl n	Gl y	Trp	Val	Asn	Trp	Leu	Met	
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	ggc	t ac	ct c	aac	t gc	caa	gt c	at c	cac	cac	ct c	t t t	ccg	agc	at g	ccg	1200
	Gl y	Tyr	Leu	Asn	Cys	Gl n	Val	I l e	Hi s	Hi s	Leu	Phe	Pro	Ser	Met	Pro	
				385		390					395				400		
	cag	t t c	cgc	cag	ccc	gag	gt a	t ct	cgc	cgc	t t c	gt c	gcc	t t t	gcg	aaa	1248
	Gl n	Phe	Arg	Gl n	Pro	Gl u	Val	Ser	Arg	Arg	Phe	Val	Al a	Phe	Al a	Lys	
				405						410				415			
	aag	t gg	aac	ct c	aac	t ac	aag	gt c	at g	acc	t ac	gcc	ggc	ggc	t gg	aag	1296
	Lys	Trp	Asn	Leu	Asn	Tyr	Lys	Val	Met	Thr	Tyr	Al a	Gl y	Al a	Trp	Lys	
				420					425					430			
	gca	acg	ct c	gga	aac	ct c	gac	aac	gt g	ggc	aag	cac	t ac	t ac	gt g	cac	1344
	Al a	Thr	Leu	Gl y	Asn	Leu	Asp	Asn	Val	Gl y	Lys	Hi s	Tyr	Tyr	Val	Hi s	
				435			440						445				
	ggc	caa	cac	t cc	gga	aag	acg	gcg	t aa								1371
	Gl y	Gl n	Hi s	Ser	Gl y	Lys	Thr	Al a									
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 <212> PRT
 <213> *Ostreococcus tauri*

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 20 25
 Al a Gl u Lys Met Gl u Pro Al a Al a Leu Al a Lys Thr Phe Al a Arg Arg
 35 40
 Tyr Val Val I l e Gl u Gl y Val Gl u Tyr Asp Val Thr Asp Phe Lys Hi s
 50 55
 Pro Gl y Gl y Thr Val I l e Phe Tyr Al a Leu Ser Asn Thr Gl y Al a Asp
 65 70 75
 Al a Thr Gl u Al a Phe Lys Gl u Phe Hi s Hi s Arg Ser Arg Lys Al a Arg
 85 90
 Lys Al a Leu Al a Al a Leu Pro Ser Arg Pro Al a Lys Thr Al a Lys Val
 100 110
 Asp Asp Al a Gl u Met Leu Gl n Asp Phe Al a Lys Trp Arg Lys Gl u Leu
 115 120
 Gl u Arg Asp Gl y Phe Phe Lys Pro Ser Pro Al a Hi s Val Al a 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 Ala Arg Tyr Val Val Ser Ser Val Leu Val Tyr Ala Cys Phe Phe Gly
 Ala Arg Cys Gly Val Trp Val Gn His Gu Gly Gly His Ser Ser Leu Thr
 Gly Asn Ile Trp Trp Asp Lys Arg Ile Gn Ala Phe Thr Ala Gly Phe
 Gly Leu Ala Gly Ser Gly Asp Met Trp Asn Ser Met His Asn Lys His
 His Ala Thr Pro Gn Lys Val Arg His Asp Met Asp Leu Asp Thr Thr
 225 Pro Ala Val Ala Phe Phe Asn Thr Ala Val Gu Asp Asn Arg Pro Arg
 Gly Phe Ser Lys Tyr Trp Leu Arg Leu Gn Ala Trp Thr Phe Ile Pro
 Val Thr Ser Gly Leu Val Leu Leu Phe Trp Met Phe Phe Leu His Pro
 Ser Lys Ala Leu Lys Gly Gly Lys Tyr Gu Gu Leu Val Trp Met Leu
 Ala Ala His Val Ile Arg Thr Trp Thr Ile Lys Ala Val Thr Gly Phe
 305 Thr Ala Met Gn Ser Tyr Gly Leu Phe Leu Ala Thr Ser Trp Val Ser
 Gly Cys Tyr Leu Phe Ala His Phe Ser Thr Ser His Thr His Leu Asp
 Val Val Pro Ala Asp Gu His Leu Ser Trp Val Arg Tyr Ala Val Asp
 His Thr Ile Asp Ile Asp Pro Ser Gn Gly Trp Val Asn Trp Leu Met
 Gly Tyr Leu Asn Cys Gn Val Ile His His Leu Phe Pro Ser Met Pro
 385 Gn Phe Arg Gn Pro Gu Val Ser Arg Arg Phe Val Ala Phe Ala Lys
 Lys Trp Asn Leu Asn Tyr Lys Val Met Thr Tyr Ala Gly Ala Trp Lys
 Ala Thr Leu Gly Asn Leu Asp Asn Val Gly Lys His Tyr Val His
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 <212> DNA
 <213> Leishmani a major

<220>
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 <222> (1)..(1254)

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 Asp Gly Val Leu Tyr Asp Cys Thr Asp Phe Arg His Pro Gly Gly Ser
 20 att ctg aaa tac tac ctg ggc agc ggc gac gcc acc gag acg tac caa 144
 Ile Leu Lys Tyr Tyr Leu Gly Ser Gly Asp Ala Thr Gu Thr Tyr Gn
 35 cag ttc cac ttg aag ctg ccc agg gcg gac aag tat ctg aag cgg ctg 192
 Gn Phe His Leu Lys Leu Pro Arg Ala Asp Lys Tyr Leu Lys Arg Leu
 50 ccg aat cgc ccg gcg ccg cca cag cac agc gtc aac gtg gat gag cag 240
 Pro Asn Arg Pro Ala Pro Pro Gn His Ser Val Asn Val Asp Gu Gn
 65 aag cga ttg gag aag ctg tgc cgg gac ttc aag gcg ctg cag gat gcg 288
 Lys Arg Leu Gu Lys Leu Ser Arg Asp Phe Lys Ala Leu Gn Asp Ala
 Sei te 68

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Cys	Val	Glu	Glu	Gly	Leu	Phe	Asn	Ala	Ser	Trp	Pro	His	Ile	Val	Tyr		
			100					105					110				
cg	ttt	tct	gag	ctg	atc	ctg	atg	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		
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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	ccg	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	ccg	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		
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agg	aac	gcg	agc	att	cg	cg	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	cg	cac	gcc	atg	cg	act	cag	cg	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	cg	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	tgc	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		
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Ile	Tyr	Thr	Met	Asn	Val	Thr	Pro	Ser	Trp	Phe	Val	Thr	Trp	Phe	Met		
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Gly	Tyr	Leu	Asn	Tyr	Gln	Val	Glu	His	His	Leu	Phe	Thr	Met	Pro			
		355				360						365					
cag	ttc	cg	ttc	gtc	caa	ctg	gcg	ccg	cga	gtg	ccg	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	tgc	cgt	ccg	tac	atg	gag	tgc	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	Gly			
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aac	t aa																1254
Asn																	

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<213> Lei shmani a maj or

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Met Ala Leu Asp Asn Val Arg Pro His Gln Pro Asn Glu Val Leu Ile
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 Asp Gly Val Leu Tyr Asp Cys Thr Asp Phe Arg His Pro Gly Gly Ser
 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
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 Asn

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<211> 401

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 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 Gu 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
 Gu 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 xaa Trp xaa xaa
 210 215 220
 xaa Gn 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 Gu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
 260 265 270

PF58307.txt

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xaa 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 xaa Tyr xaa Phe xaa xaa Phe
   290                295                300
xaa xaa xaa His xaa His xaa 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 xaa Leu xaa Tyr xaa xaa
   370                375                380
xaa xaa Tyr xaa 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

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- <222> (10)..(10)
- <223> xaa in position 10 is any amino acid
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- <222> (12)..(12)
- <223> xaa in position 12 is Phe, Leu or Val
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- <222> (21)..(21)
- <223> xaa in position 21 is any amino acid
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- <223> xaa in position 22 is Lys or Arg
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- <221> Variant
- <222> (23)..(23)
- <223> xaa in position 23 is Ile or Met
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 xaa Trp xaa Asp 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

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Val xaa Trp xaa Met xaa xaa xaa Asn xaa xaa xaa Gln xaa xaa His
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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
    
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<222> (8)..(8)

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<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

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<221> Variant

<222> (17)..(17)

<223> xaa in position 17 is any amino acid

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<222> (18)..(18)

<223> xaa in position 18 is any or no amino acid

<220>

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<222> (20)..(20)

<223> xaa in position 20 is Phe, Ile or Leu

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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
    
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<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>
<221> Variant
<222> (45)..(45)
<223> xaa in position 45 is any amino acid
<220>
<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 xaa
 1 5 10 15
 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)

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<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

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<210> 63
<211> 28
<212> PRT
<213> Artificial sequence

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<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

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<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 Gu or Gn
<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

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```

<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

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<210> 64
<211> 903
<212> DNA
<213> Ostreococcus tauri

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<220>
<221> CDS
<222> (1)..(903)

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<400> 64
atg tct gct tct gga gct ttg ttg cct gct att gct ttc gct gct tac
Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
 1           5           10           15

```

PF58307.txt

gct t ac gct acc t ac gct t at gct t t c gag t gg t ct cat gct aac gga 96
Al a Tyr Al a Thr Tyr Al a Tyr Al a Phe Gu Trp Ser Hi s Al a Asn Gly
20 25 30
at c gat aac gt g gat gct aga gag t gg gct t t g t ct t t g aga 144
I le Asp Asn Val Asp Al a Arg Gu Trp I le Gly Al a Leu Ser Leu Arg
35 40 45
ct c cct gca att gct acc acc at g t ac ct c t t g t t c t gc ct t gt g gga 192
Leu Pro Al a I le Al a Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
50 55
cct aga t t g at g gct aag agg gag gct t t t gat cct aag gga t t c at g 240
Pro Arg Leu Met Al a Lys Arg Gu Al a Phe Asp Pro Lys Gly Phe Met
65 70 80
ct c gct t ac aac gct t ac caa acc gct t t c aac gt t gt g gt g ct c gga 288
Leu Al a Tyr Asn Al a Tyr Gn Thr Al a Phe Asn Val Val Val Leu Gly
85 90 95
at g t t c gct aga gag at c t ct gga t t g gga caa cct gt t t gg gga t ct 336
Met Phe Al a Arg Gu I le Ser Gly Leu Gly Gn Pro Val Trp Gly Ser
100 105 110
act at g cct t gg agc gat agg aag t cc t t c aag at t t t g t t g gga gt g 384
Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys I le Leu Leu Gly Val
115 120 125
t gg ct c cat t ac aac aat aag t ac ct c gag t t g t t g gat act gt g t t c 432
Trp Leu Hi s Tyr Asn Asn Lys Tyr Leu Gu Leu Leu Asp Thr Val Phe
130 135 140
at g gt g gct agg aaa aag acc aag cag ct c t ct t t c t t g cat gt g t ac 480
Met Val Al a Arg Lys Lys Thr Lys Gn Leu Ser Phe Leu Hi s Val Tyr
145 150 160
cat cat gct t t g t t g at t t gg gct t gg t gg ct t gt t t gt cat ct c at g 528
Hi s Hi s Al a Leu Leu I le Trp Al a Trp Trp Leu Val Cys Hi s Leu Met
165 170 175
gct acc aac gat t gc at c gat gct t at t t c gga gct gct t gc aac t ct 576
Al a Thr Asn Asp Cys I le Asp Al a Tyr Phe Gly Al a Al a Cys Asn Ser
180 185 190
t t c at c cac at c gt g at g t ac t cc t ac t ac ct c at g t ct gct t t g gga 624
Phe I le Hi s I le Val Met Tyr Ser Tyr Tyr Leu Met Ser Al a Leu Gly
195 200 205
at t aga t gc cct t gg aag aga t at at c acc cag gct cag at g t t g caa 672
I le Arg Cys Pro Trp Lys Arg Tyr I le Thr Gn Al a Gn Met Leu Gn
210 215 220
t t c gt g at c gt g t t c gct cat gct gt t t t c gt g ct c aga caa aag cac 720
Phe Val I le Val Phe Al a Hi s Al a Val Phe Val Leu Arg Gn Lys Hi s
225 230 235 240
t gc cct gt t act t t g cct t gg gca caa at g t t c gt g at g aca aat at g 768
Cys Pro Val Thr Leu Pro Trp Al a Gn Met Phe Val Met Thr Asn Met
245 250 255
t t g gt g ct c t t c gga aac t t c t ac ct c aag gct t ac t ct aac aag t ct 816
Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Al a Tyr Ser Asn Lys Ser
260 265 270
agg gga gat gga gct t ct t ct gt t aag cct gct gag act act aga gca 864
Arg Gly Asp Gly Al a Ser Ser Val Lys Pro Al a Gu Thr Thr Arg Al a
275 280 285
cct t ct gt g aga aga acc agg t cc agg aag at c gat t ga 903
Pro Ser Val Arg Arg Thr Arg Ser Arg Lys I le Asp
290 295 300

<210> 65
<211> 300
<212> PRT
<213> *Ostreococcus tauri*

<400> 65
Met Ser Al a Ser Gly Al a Leu Leu Pro Al a I le Al a Phe Al a Al a Tyr
1 5 10 15
Al a Tyr Al a Thr Tyr Al a Tyr Al a Phe Gu Trp Ser Hi s Al a Asn Gly
20 25 30
I le Asp Asn Val Asp Al a Arg Gu Trp I le Gly Al a Leu Ser Leu Arg
35 40 45
Leu Pro Al a I le Al a Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
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PF58307. txt

50 55 60
 Pro Arg Leu Met Ala Lys Arg Gu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75 80
 Leu Ala Tyr Asn Ala Tyr Gn Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95
 Met Phe Ala Arg Gu Ile Ser Gly Leu Gly Gn 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 Gu Leu Leu Asp Thr Val Phe
 130 135 140
 Met Val Ala Arg Lys Lys Thr Lys Gn 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 Gn Ala Gn Met Leu Gn
 210 215 220
 Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gn Lys His
 225 230 235 240
 Cys Pro Val Thr Leu Pro Trp Ala Gn 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 Gu 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 ct t ct a gt g ct c t cg gcc gct cgc cag 48
 Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gn
 1 5 10 15
 agc ttc cag cag gac att gac aac ccc aac ggg gcc tac t cg acc t cg 96
 Ser Phe Gn Gn Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
 20 25 30
 tgg act ggc ctg ccc att gtg atg t ct gt g gt c t at ct c agc ggt gt g 144
 Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
 35 40 45
 ttt ggg ct c aca aag tac ttc gag aac cgg aag ccc atg acg ggg ct g 192
 Phe Gly Leu Thr Lys Tyr Phe Gu Asn Arg Lys Pro Met Thr Gly Leu
 50 55 60
 aag gac tac atg ttc act tac aat ct c tac cag gt g at c at c aac gt g 240
 Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gn Val Ile Ile Asn Val
 65 70 75 80
 tgg tgc gt g gt g gcc ttt ct c ct g gag gt g cgg cgt gcg ggc atg t ca 288
 Trp Cys Val Val Ala Phe Leu Leu Gu Val Arg Arg Ala Gly Met Ser
 85 90 95
 ct c at c ggc aat aag gt g gac ct t ggg ccc aac t cc t t c agg ct c ggc 336
 Leu Ile Gly Asn Lys Val Asp Leu Gly Pro Asn Ser Phe Arg Leu Gly
 100 105 110
 ttc gt c acg tgg gt g cac tac aac aac aag tac gt g gag ct c ct c gac 384
 Phe Val Thr Trp Val His Tyr Asn Asn Lys Tyr Val Gu Leu Leu Asp
 115 120 125
 acc ct a tgg atg gt g ct g cgc aag aag acg cag cag gt c t cc t t c ct c 432
 Thr Leu Trp Met Val Leu Arg Lys Lys Thr Gn Gn Val Ser Phe Leu

PF58307. txt

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130          135          140
cac gt c t at cat cac gt g ct t ct g at g t gg gcc t gg t t c gt t gt c gt c      480
His Val Tyr His His Val Leu Leu Met Tr p Ala Tr p Phe Val Val Val Val
145          150          155
aag ct c ggc aat ggt gac gca t at t t t ggc ggt ct c at g aac t cg      528
Lys Leu Gly Asn Gly Gly Asp Ala Tyr Phe Gly Gly Leu Met Asn Ser
165          170          175
at c at c cac gt g at g at g t at t cc t ac t ac acc at g gcg ct c ct g ggc      576
Ile Ile His Val Met Met Tyr Ser Tyr Tyr Thr Met Ala Leu Leu Gly
180          185          190
t gg t ca t gc ccc t gg aag cgc t ac ct c acg cag gca cag ct c gt g cag      624
Tr p Ser Cys Pro Tr p Lys Arg Tyr Leu Thr Gl n Ala Gl n Leu Val Gl n
195          200          205
t t t t gc at c t gc ct c gcc cac t cc aca t gg gcg gca gt a acg ggt gcc      672
Phe Cys Ile Cys Leu Ala His Ser Thr Tr p Ala Ala Val Thr Gly Ala
210          215          220
t ac ccg t gg cga att t gc t t g gt g gag gt g t gg gt g at g gt g t cc at g      720
Tyr Pro Tr p Arg Ile Cys Leu Val Gl u Val Tr p Val Met Val Ser Met
225          230          235
ct g gt g ct c t t c aca cgc t t c t ac cgc cag gcc t at gcc aag gag gcg      768
Leu Val Leu Phe Thr Arg Phe Tyr Arg Gl n Ala Tyr Ala Lys Gl u Ala
245          250          255
aag gcc aag gag gcg aaa aag ct c gca cag gag gca t ca cag gcc aag      816
Lys Ala Lys Gl u Ala Lys Lys Leu Ala Gl n Gl u Ala Ser Gl n Ala Lys
260          265          270
gcg gt c aag gcg gag t aa
Ala Val Lys Ala Gl u
275

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<210> 67
 <211> 277
 <212> PRT
 <213> Pavlova sp

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<400> 67
Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gl n
1          5          10          15
Ser Phe Gl n Gl n Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
20          25          30
Tr p Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
35          40          45          50
Phe Gly Leu Thr Lys Tyr Phe Gl u Asn Arg Lys Pro Met Thr Gly Leu
55          60          65
Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gl n Val Ile Ile Asn Val
70          75          80
Tr p Cys Val Val Ala Phe Leu Leu Gl u 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 Tr p Val His Tyr Asn Asn Lys Tyr Val Gl u Leu Leu Asp
115          120          125
Thr Leu Tr p Met Val Leu Arg Lys Lys Thr Gl n Gl n Val Ser Phe Leu
130          135          140
His Val Tyr His His Val Leu Leu Met Tr p Ala Tr p Phe Val Val Val
145          150          155
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
Tr p Ser Cys Pro Tr p Lys Arg Tyr Leu Thr Gl n Ala Gl n Val Gl n
195          200          205
Phe Cys Ile Cys Leu Ala His Ser Thr Tr p Ala Ala Val Thr Gly Ala
210          215          220
Tyr Pro Tr p Arg Ile Cys Leu Val Gl u Val Tr p Val Met Val Ser Met
225          230          235
Leu Val Leu Phe Thr Arg Phe Tyr Arg Gl n Ala Tyr Ala Lys Gl u Ala
245          250          255
Lys Ala Lys Gl u Ala Lys Lys Leu Ala Gl n Gl u Ala Ser Gl n Ala Lys

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Sei te 88

Al a Val Lys Ala Gl u
275

<210> 68
<211> 1077
<212> DNA
<213> Thal assi osi r a pseudonana

<220>
<221> CDS
<222> (1).. (1077)

<400> 68
at g tgc tca tca ccg ccg tca caa tcc aaa aca aca tcc ct c ct a gca 48
Met Cys Ser Ser Pro Pro Ser Gl n Ser Lys Thr Thr Ser Leu Leu Ala
1 5 10 15
cgg tac acc acc gcc gcc ct c ct c ct c acc ct c aca aca tgg tgc 96
Arg Tyr Thr Thr Ala Ala Leu Leu Leu Thr Leu Thr Thr Trp Cys
20 25 30
cac ttc gcc ttc cca gcc gcc acc gcc aca ccc ggc ct c acc gcc gaa 144
His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Gl u
35 40 45
at g cac tcc tac aaa gt c cca ct c ggt ct c acc gt a ttc tac ct g ct g 192
Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu
50 55 60
agt ct a ccg tca ct a aag t ac gt t acg gac aac t ac ct t gcc aaa aag 240
Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys
65 70 75 80
t at gat at g aag tca ct c ct a acg gaa tca at g gt g tt g tac aat gt g 288
Tyr Asp Met Lys Ser Leu Leu Thr Gl u Ser Met Val Leu Tyr Asn Val
85 90 95
gcg caa gt g ct g ct c aat ggg tgg acg gt g t at gcg at t gt g gat gcg 336
Ala Gl n Val Leu Leu Asn Gly Trp Thr Val Tyr Ala Ile Val Asp Ala
100 105 110
gt g at g aat aga gac cat ccg ttt at t gga agt aga agt tt g gt t ggg 384
Val Met Asn Arg Asp His Pro Phe Ile Gly Ser Arg Ser Leu Val Gly
115 120 125
gcg gcg tt g cat agt ggg agc t cg t at gcg gt g t gg gt t cat t at t gt 432
Ala Ala Leu His Ser Gly Ser Ser Tyr Ala Val Trp Val His Tyr Cys
130 140
gat aag t at tt g gag ttc ttt gat acg t at ttt at g gt g tt g agg ggg 480
Asp Lys Tyr Leu Gl u Phe Phe Asp Thr Tyr Phe Met Val Leu Arg Gly
145 150 155 160
aaa at g gac cag gt c tcc ttc ct c cac at c tac cac cac acg acc at a 528
Lys Met Asp Gl n Val Ser Phe Leu His Ile Tyr His His Thr Thr Ile
165 170 175
gcg tgg gca tgg tgg at c gcc ct c cgc ttc tcc ccc ggt gga gac at t 576
Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly Gly Asp Ile
180 185 190
t ac ttc ggg gca ct c ct c aac tcc at c cac gt c ct c at g t at tcc 624
Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu Met Tyr Ser
195 200 205
t ac tac gcc ct t gcc ct a ct c aag gt c agt tgt cca tgg aaa cga tac 672
Tyr Tyr Ala Leu Ala Leu Lys Val Ser Cys Cys Pro Trp Lys Arg Tyr
210 215 220
ct g act caa gct caa tta tt g caa ttc aca agt gt g gt g gt t t at acg 720
Leu Thr Gl n Ala Gl n Leu Leu Gl n Phe Thr Ser Val Val Val Tyr Thr
225 230 235 240
ggg tgt acg ggt t at act cat tac t at 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 t at t at ttc tgt tgt gga gt g cag 816
Gl u Thr Gl n Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gl n
260 265 270
gt g ttt gag at g gt t agt tt g ttt gt a ct c ttt tcc at c ttt t at aaa 864
Val Phe Gl u Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
275 280 285

PF58307. txt

cga t cc t at t cg aag aag aac aag t ca gga gga aag gat agc aag aag 912
 Arg Ser Tyr Ser Lys Lys Asn Lys Ser G y G y Lys Asp Ser Lys Lys
 290 295 300
 aat gat gat ggg aat aat gag gat caa t gt cac aag gct at g aag gat 960
 Asn Asp Asp G y Asn Asn G u Asp G n Cys Hi s Lys Al a M et Lys Asp
 305 310 315 320
 at a t cg gag ggt gcg aag gag gt t gt g ggg cat gca gcg aag gat gct 1008
 Ile Ser G u G y Al a Lys G u Val Val G y Hi s Al a Al a Lys Asp Al a
 325 330 335
 gga aag t t g gt g gct acg gcg agt aag gct gt a aag agg aag gga act 1056
 G y Lys Leu Val Al a Thr Al a Ser Lys Al a Val Lys Arg Lys G y Thr
 340 345 350
 cgt gt t act ggt gcc at g t ag 1077
 Arg Val Thr G y Al a M et

<210> 69

<211> 358

<212> PRT

<213> Thal assi osi r a pseudonana

<400> 69

M et Cys Ser Ser Pro Pro Ser G n Ser Lys Thr Thr Ser Leu Leu Al a
 1 5 10 15
 Arg Tyr Thr Thr Al a Al a Leu Leu Leu Leu Thr Leu Thr Thr Tr p Cys
 20 25
 Hi s Phe Al a Phe Pro Al a Al a Thr Al a Thr Pro G y Leu Thr Al a G u
 35 40
 M et Hi s Ser Tyr Lys Val Pro Leu G y Leu Thr Val Phe Tyr Leu Leu
 50 55 60
 Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Al a Lys Lys
 65 70 75 80
 Tyr Asp M et Lys Ser Leu Leu Thr G u Ser M et Val Leu Tyr Asn Val
 85 90 95
 Al a G n Val Leu Leu Asn G y Tr p Thr Val Tyr Al a Ile Val Asp Al a
 100 105
 Val M et Asn Arg Asp Hi s Pro Phe Ile G y Ser Arg Ser Leu Val G y
 115 120 125
 Al a Al a Leu Hi s Ser G y Ser Ser Tyr Al a Val Tr p Val Hi s Tyr Cys
 130 135 140
 Asp Lys Tyr Leu G u Phe Phe Asp Thr Tyr Phe M et Val Leu Arg G y
 145 150 155 160
 Lys M et Asp G n Val Ser Phe Leu Hi s Ile Tyr Hi s Hi s Thr Thr Ile
 165 170 175
 Al a Tr p Al a Tr p Tr p Ile Al a Leu Arg Phe Ser Pro G y G y Asp Ile
 180 185 190
 Tyr Phe G y Al a Leu Leu Asn Ser Ile Ile Hi s Val Leu M et Tyr Ser
 195 200 205
 Tyr Tyr Al a Leu Al a Leu Leu Lys Val Ser Cys Pro Tr p Lys Arg Tyr
 210 215 220
 Leu Thr G n Al a G n Leu Leu G n Phe Thr Ser Val Val Tyr Thr
 225 230 235 240
 G y Cys Thr G y Tyr Thr Hi s Tyr Tyr Hi s Thr Lys Hi s G y Al a Asp
 245 250 255
 G u Thr G n Pro Ser Leu G y Thr Tyr Tyr Phe Cys Cys G y Val G n
 260 265 270
 Val Phe G u M et Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
 275 280 285
 Arg Ser Tyr Ser Lys Lys Asn Lys Ser G y G y Lys Asp Ser Lys Lys
 290 295 300
 Asn Asp Asp G y Asn Asn G u Asp G n Cys Hi s Lys Al a M et Lys Asp
 305 310 315 320
 Ile Ser G u G y Al a Lys G u Val Val G y Hi s Al a Al a Lys Asp Al a
 325 330 335
 G y Lys Leu Val Al a Thr Al a Ser Lys Al a Val Lys Arg Lys G y Thr
 340 345 350
 Arg Val Thr G y Al a M et 355

<210> 70
 <211> 903
 <212> DNA
 <213> *Ostreococcus tauri*

<220>
 <221> CDS
 <222> (1)..(903)

<400> 70
 at g agc gcc t cc ggt gcg ct g ct g ccc gcg at c gcg t cc gcc gcg t ac 48
 Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Ser Ala Ala Tyr
 1 5 10 15
 gcg t ac gcg acg t ac gcc t ac gcc t t t gag t gg t cg cac gcg aat ggc 96
 Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Gu Trp Ser His Ala Asn Gly
 20 25 30
 at c gac aac gt c gac gcg cgc gag t gg at c ggt gcg ct g t cg t t g agg 144
 Ile Asp Asn Val Asp Ala Arg Gu Trp Ile Gly Ala Leu Ser Leu Arg
 35 40 45
 ct c ccg gcg at c gcg acg acg at g t ac ct g t t g t t c t gc ct g gt c gga 192
 Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
 50 55 60
 ccg agg t t g at g gcg aag cgc gag gcg t t c gac ccg aag ggg t t c at g 240
 Pro Arg Leu Met Ala Lys Arg Gu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75 80
 ct g gcg t ac aat gcg t at cag acg gcg t t c aac gt c gt c gt g ct c ggg 288
 Leu Ala Tyr Asn Ala Tyr Gn Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95
 at g t t c gcg cga gag at c t cg ggg ct g ggg cag ccc gt g t gg ggg t ca 336
 Met Phe Ala Arg Gu Ile Ser Gly Leu Gly Gn Pro Val Trp Gly Ser
 100 105 110
 acc at g ccg t gg agc gat aga aaa t cg t t t aag at c ct c ct c ggg gt g 384
 Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
 115 120 125
 t gg t t g cac t ac aac aac aaa t at t t g gag ct a t t g gac act gt g t t c 432
 Trp Leu His Tyr Asn Asn Lys Tyr Leu Gu Leu Leu Asp Thr Val Phe
 130 135 140 145
 at g gt t gcg cgc aag aag acg aag cag t t g agc t t c t t g cac gt t t at 480
 Met Val Ala Arg Lys Lys Thr Lys Gn Leu Ser Phe Leu His Val Tyr
 150 155 160
 cat cac gcc ct g t t g at c t gg gcg t gg t gg t t g gt g t gt cac t t g at g 528
 His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
 165 170 175
 gcc acg aac gat t gt at c gat gcc t ac t t c ggc gcg gcg t gc aac t cg 576
 Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
 180 185 190
 t t c att cac at c gt g at g t ac t cg t at t at ct c at g t cg gcg ct c ggc 624
 Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
 195 200 205
 at t cga t gc ccg t gg aag cga t ac at c acc cag gct caa at g ct c caa 672
 Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gn Ala Gn Met Leu Gn
 210 215 220
 t t c gt c att gt c t t c gcg cac gcc gt g t t c gt g ct g cgt cag aag cac 720
 Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gn Lys His
 225 230 235 240
 t gc ccg gt c acc ct t cct t gg gcg caa at g t t c gt c at g acg aac at g 768
 Cys Pro Val Thr Leu Pro Trp Ala Gn Met Phe Val Met Thr Asn Met
 245 250 255
 ct c gt g ct c t t c ggg aac t t c t ac ct c aag gcg t ac t cg aac aag t cg 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 t cc gt g aaa cca gcc gag acc acg cgc gcg 864
 Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Gu Thr Thr Arg Ala
 275 280 285
 ccc agc gt g cga cgc acg cga t ct cga aaa at t gac t aa 903
 Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
 290 295 300

<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 Gu Trp Ser His Ala Asn Gly
 20 25 30
 Ile Asp Asn Val Asp Ala Arg Gu 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 Gu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75 80
 Leu Ala Tyr Asn Ala Tyr Gn Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95
 Met Phe Ala Arg Gu Ile Ser Gly Leu Gly Gn 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 Gu Leu Leu Asp Thr Val Phe
 130 135 140
 Met Val Ala Arg Lys Lys Thr Lys Gn 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 Gn Ala Gn Met Leu Gn
 210 215 220
 Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gn Lys His
 225 230 235 240
 Cys Pro Val Thr Leu Pro Trp Ala Gn 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 Gu 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)
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 <222> (30)..(43)
 <223> xaa in position 30 to 43 is any or no amino acid
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 <222> (45)..(50)
 <223> xaa in position 45 to 50 is any amino acid
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 <222> (53)..(73)
 <223> xaa in position 53 to 73 is any amino acid

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<223> xaa in position 74 to 79 is any or no amino acid
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<222> (81)..(82)
<223> xaa in position 81 to 82 is any amino acid
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<221> Variant
<222> (85)..(86)
<223> xaa in position 85 to 86 is any amino acid
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<221> Variant
<222> (88)..(90)
<223> xaa in position 88 to 90 is any amino acid
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<222> (92)..(110)
<223> xaa in position 92 to 110 is any amino acid
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<222> (112)..(127)
<223> xaa in position 112 to 127 is any amino acid
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<222> (128)..(128)
<223> xaa in position 128 is any or no amino acid
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<222> (130)..(130)
<223> xaa in position 130 is any amino acid
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<222> (133)..(134)
<223> xaa in position 133 to 134 is any amino acid
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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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<222> (143)..(144)
<223> xaa in position 143 to 144 is any amino acid
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<222> (147)..(147)
<223> xaa in position 147 is any amino acid
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<222> (149)..(149)
<223> xaa in position 149 is any amino acid
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<222> (151)..(152)
<223> xaa in position 151 to 152 is any amino acid
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<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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<221> Variant
<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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<221> Variant
<222> (184)..(184)
<223> xaa in position 184 is any amino acid
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<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
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<221> Variant
<222> (196)..(197)
<223> xaa in position 196 to 197 is any amino acid
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<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
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<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

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<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 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 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 G n 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 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 G u xaa xaa Asp Thr xaa xaa
  130     135     140
Met Val xaa Arg xaa Lys xaa xaa G n 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 G y xaa 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 G n Ala G n xaa xaa G n
  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

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<210> 73
<211> 45
<212> PRT
<213> Artificial sequence

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<220>
<221> Variant
<222> (4)..(4)

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<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

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<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
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

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<210> 75
<211> 37
<212> PRT
<213> Artificial sequence

<220>
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<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 Gu or Lys
<220>

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<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>
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 <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 Gu or Gly
 <220>
 <221> Variant
 <222> (25)..(29)
 <223> xaa in position 25 to 29 is any amino acid
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 <221> Variant
 <222> (30)..(30)
 <223> xaa in position 30 is Asn, Pro or Gn
 <220>
 <221> Variant
 <222> (31)..(32)
 <223> xaa in position 31 to 32 is Ala, Asp or Gu
 <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, Gn 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
 20 25 30
 xaa xaa xaa xaa xaa
 35

<210> 76
 <211> 19
 <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)..(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
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 <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
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 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 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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gt a	at g	cgc	gag	cgc	gt c	gt g	gct	cgg	ct c	aag	gag	cgc	ggc	aag	gcc	384
Val	Met	Arg	Gl u	Arg	Val	Val	Al a	Arg	Leu	Lys	Gl u	Arg	Gl y	Lys	Al a	
		115					120					125				
cgc	cgc	gga	ggc	t ac	gag	ct c	t gg	at c	aag	gcg	t t c	ct g	ct g	ct c	gt c	432
Arg	Arg	Gl y	Gl y	Tyr	Gl u	Leu	Tr p	I l e	Lys	Al a	Phe	Leu	Leu	Leu	Val	
		130					135				140					
ggc	t t c	t gg	agc	t cg	ct g	t ac	t gg	at g	t gc	acg	ct g	gac	ccc	t cg	t t c	480
Gl y	Phe	Tr p	Ser	Ser	Leu	Tyr	Tr p	Met	Cys	Thr	Leu	Asp	Pro	Ser	Phe	
		145			150					155					160	
ggg	gcc	at c	ct g	gcc	gcc	at g	t cg	ct g	ggc	gt c	t t t	gcc	gcc	t t t	gt g	528
Gl y	Al a	I l e	Leu	Al a	Al a	Met	Ser	Leu	Gl y	Val	Phe	Al a	Al a	Phe	Val	
				165				170						175		
ggc	acg	t gc	at c	cag	cac	gac	ggc	aac	cac	ggc	gcc	t t t	gcc	cag	t cg	576
Gl y	Thr	Cys	I l e	Gl n	Hi s	Asp	Gl y	Asn	Hi s	Gl y	Al a	Phe	Al a	Gl n	Ser	
			180					185						190		
cga	t gg	gt c	aac	aag	gt t	gcc	ggg	t gg	acg	ct c	gac	at g	at c	ggc	gcc	624
Arg	Tr p	Val	Asn	Lys	Val	Al a	Gl y	Tr p	Thr	Leu	Asp	Met	I l e	Gl y	Al a	
			195				200					205				
agc	ggc	at g	acg	t gg	gag	t t c	cag	cac	gt c	ct g	ggc	cac	cat	ccg	t ac	672
Ser	Gl y	Met	Thr	Tr p	Gl u	Phe	Gl n	Hi s	Val	Leu	Gl y	Hi s	Hi s	Pro	Tyr	
		210				215					220					
acg	aac	ct g	at c	gag	gag	gag	aac	ggc	ct g	caa	aag	gt g	agc	ggc	aag	720
Thr	Asn	Leu	I l e	Gl u	Gl u	Gl u	Asn	Gl y	Leu	Gl n	Lys	Val	Ser	Gl y	Lys	
		225		230						235					240	
aag	at g	gac	acc	aag	ct g	gcc	gac	cag	gag	agc	gat	ccg	gac	gt c	t t t	768
Lys	Met	Asp	Thr	Lys	Leu	Al a	Asp	Gl n	Gl u	Ser	Asp	Pro	Asp	Val	Phe	
			245					250						255		
t cc	acg	t ac	ccg	at g	cgc	ct g	cac	ccg	t gg	cac	cag	aag	cgc	t gg		816
Ser	Thr	Tyr	Pro	Met	Met	Arg	Leu	Hi s	Pro	Tr p	Hi s	Gl n	Lys	Arg	Tr p	
			260					265					270			
t ac	cac	cgt	t t c	cag	cac	at t	t ac	ggc	ccc	t t c	at c	t t t	ggc	t t c	at g	864
Tyr	Hi s	Arg	Phe	Gl n	Hi s	I l e	Tyr	Gl y	Pro	Phe	I l e	Phe	Gl y	Phe	Met	
		275					280					285				
acc	at c	aac	aag	gt g	gt c	acg	cag	gac	gt c	ggt	gt g	gt g	ct c	cgc	aag	912
Thr	I l e	Asn	Lys	Val	Val	Thr	Gl n	Asp	Val	Gl y	Val	Val	Leu	Arg	Lys	
		290				295					300					
cgg	ct c	t t c	cag	at t	gac	gcc	gag	t gc	cgg	t ac	gcg	agc	cca	at g	t ac	960
Arg	Leu	Phe	Gl n	I l e	Asp	Al a	Gl u	Cys	Arg	Tyr	Al a	Ser	Pro	Met	Tyr	
		305			310					315					320	
gt g	gcg	cgt	t t c	t gg	at c	at g	aag	gcg	ct c	acg	gt g	ct c	t ac	at g	gt g	1008
Val	Al a	Arg	Phe	Tr p	I l e	Met	Lys	Al a	Leu	Thr	Val	Leu	Tyr	Met	Val	
				325					330					335		
gcc	ct g	ccg	t gc	t ac	at g	cag	ggc	ccg	t gg	cac	ggc	ct c	aag	ct g	t t c	1056
Al a	Leu	Pro	Cys	Tyr	Met	Gl n	Gl y	Pro	Tr p	Hi s	Gl y	Leu	Lys	Leu	Phe	
			340					345					350			
gcg	at c	gcg	cac	t t t	acg	t gc	ggc	gag	gt g	ct c	gca	acc	at g	t t c	at t	1104
Al a	I l e	Al a	Hi s	Phe	Thr	Cys	Gl y	Gl u	Val	Leu	Al a	Thr	Met	Phe	I l e	
		355				360						365				
gt g	aac	cac	at c	at c	gag	ggc	gt c	t cg	t ac	gct	t cc	aag	gac	gcg	gt c	1152
Val	Asn	Hi s	I l e	I l e	Gl u	Gl y	Val	Ser	Tyr	Al a	Ser	Lys	Asp	Al a	Val	
		370				375					380					
aag	ggc	acg	at g	gcg	ccg	ccg	aag	acg	at g	cac	ggc	gt g	acg	ccc	at g	1200
Lys	Gl y	Thr	Met	Al a	Pro	Pro	Lys	Thr	Met	Hi s	Gl y	Val	Thr	Pro	Met	
		385			390					395					400	
aac	aac	acg	cgc	aag	gag	gt g	gag	gcg	gag	ct c	aag	t ct	ggc	ggc		1248
Asn	Asn	Thr	Arg	Lys	Gl u	Val	Gl u	Al a	Gl u	Al a	Ser	Lys	Ser	Gl y	Al a	
				405					410					415		
gt g	gt c	aag	t ca	gt c	ccg	ct c	gac	gac	t gg	gcc	gcc	gt c	cag	t gc	cag	1296
Val	Val	Lys	Ser	Val	Pro	Leu	Asp	Asp	Tr p	Al a	Al a	Val	Gl n	Cys	Gl n	
			420					425					430			
acc	t cg	gt g	aac	t gg	agc	gt c	ggc	t cg	t gg	t t c	t gg	aat	cac	t t t	t cc	1344
Thr	Ser	Val	Asn	Tr p	Ser	Val	Gl y	Ser	Tr p	Phe	Tr p	Asn	Hi s	Phe	Ser	
		435					440				445					
ggc	ggc	ct c	aac	cac	cag	at t	gag	cac	cac	ct g	t t c	ccc	ggg	ct c	agc	1392
Gl y	Gl y	Leu	Asn	Hi s	Gl n	I l e	Gl u	Hi s	Hi s	Leu	Phe	Pro	Gl y	Leu	Ser	
		450				455					460					
cac	gag	acg	t ac	t ac	cac	at c	cag	gac	gt c	gt t	cag	t cc	acc	t gc	gcc	1440
Hi s	Gl u	Thr	Tyr	Tyr	Hi s	I l e	Gl n	Asp	Val	Val	Gl n	Ser	Thr	Cys	Al a	

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465					470					475					480	
gag	t ac	ggc	gt c	ccg	t ac	cag	cac	gag	cct	t cg	ct c	t gg	acc	gcg	t ac	1488
Gu	Tyr	Gly	Val	Pro	Tyr	Gln	His	Gu	Pro	Ser	Leu	Trp	Thr	Ala	Tyr	
				485					490					495		
t gg	aag	at g	ct c	gag	cac	ct c	cgt	cag	ct c	ggc	aat	gag	gag	acc	cac	1536
Trp	Lys	Met	Leu	Gu	His	Leu	Arg	Gln	Leu	Gly	Asn	Gu	Gu	Thr	His	
			500					505					510			
gag	t cc	t gg	cag	cgc	gct	gcc	t ga									1560
Gu	Ser	Trp	Gln	Arg	Ala	Ala										
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 <211> 519
 <212> PRT
 <213> *Tr aust ochyt ri um sp.*

<400> 78

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His	Asn	Lys	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	His	Val	Tyr
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Asp	Val	Thr	Lys	Phe	Ala	Ser	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Leu
		35					40					45			
Leu	Ala	Ala	Gly	Lys	Gu	Ala	Thr	Val	Leu	Tyr	Gu	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	Ala	Asn	Gu	Lys	Gu	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
			100					105					110		
Val	Met	Arg	Gu	Arg	Val	Val	Ala	Arg	Leu	Lys	Gu	Arg	Gly	Lys	Ala
		115					120					125			
Arg	Arg	Gly	Gly	Tyr	Gu	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	Gu	Phe	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr
	210					215					220				
Thr	Asn	Leu	Ile	Gu	Gu	Asn	Gly	Leu	Gln	Lys	Val	Ser	Gly	Lys	
225				230					235					240	
Lys	Met	Asp	Thr	Lys	Leu	Ala	Asp	Gln	Gu	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	Gu	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	Gu	Val	Leu	Ala	Thr	Met	Phe	Ile
		355					360					365			
Val	Asn	His	Ile	Ile	Gu	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
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Asn	Asn	Thr	Arg	Lys	Gu	Val	Gu	Ala	Gu	Ala	Ser	Lys	Ser	Gly	Ala

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Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val Gln Cys Gln
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Thr Ser Val Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
420 425 430
Gly Gly Leu Asn His Gln Ile Gu His His Leu Phe Pro Gly Leu Ser
435 440 445
His Gu Thr Tyr Tyr His Ile Gln Asp Val Val Gln Ser Thr Cys Ala
450 455 460 465
Gu Tyr Gly Val Pro Tyr Gln His Gu Pro Ser Leu Trp Thr Ala Tyr
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490 495 500 505
Gu Ser Trp Gln Arg Ala Ala
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 <213> Thr aust ochyt r i u m sp

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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 Gu 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
ccg gac ggc caa ggc ggc gcg aac gag aag gaa aag cgg acg ctg tcg 288
Pro Asp Gly Gln Gly Gly Ala Asn Gu Lys Gu Lys Arg Thr Leu Ser
85 90 95
ggc ctg tcg tcg gcc tcg tac tac acg tgg aac agc gac ttt tac agg 336
Gly Leu 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 ctg aag gag cgc ggc aag gcc 384
Val Met Arg Gu Arg Val Val Ala Arg Leu Lys Gu Arg Gly Lys Ala
115 120 125
cgc cgc gga ggc tac gag ctg tgg atc aag gcg ctg ctg ctg ctg gtc 432
Arg Arg Gly Gly Tyr Gu 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
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 ctg 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 Gu Phe Gln His Ala Leu Gly His His Pro Tyr
210 215 220

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 aag at g gac acc aag ct g gcc gac cag gag agc gat ccg gac gt c ttt 768
 Lys Met Asp Thr Lys Leu Ala Asp Gl n Gl u Ser Asp Pro Asp Val ttt
 245 250 255
 t cc acg t ac ccg at g at g cgc ct g cac ccg t gg cac cag aag cgc t gg 816
 Ser Thr Tyr Pro Met Met Arg Leu His Pro Tr p His Gl n Lys Arg Tr p
 260 265 270
 t ac cac cgt ttc cag cac att t ac ggc ccc ttc at c ttt ggc ttc at g 864
 Tyr His Arg Phe Gl n His Ile Tyr Gy Pro Phe Ile Phe Gy Phe Met
 275 280 285
 acc at c aac aag gt g gt c acg cag gac gt c ggt gt g gt g ttc cgc aag 912
 Thr Ile Asn Lys Val Val Thr Gl n Asp Val Gy Val Val Phe Arg Lys
 290 295 300
 cgg ct c ttc cag att gac gcc gag tgc cgg t ac gcg agc cca at g t ac 960
 Arg Leu Phe Gl n Ile Asp Ala Gl u Cys Arg Tyr Ala Ser Pro Met Tyr
 305 310 315 320
 gt g gcg cgt ttc tgg at c at g aag gcg ct c acg gt g ct c t ac at g gt g 1008
 Val Ala Arg Phe Tr p Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
 325 330 335
 gcc ct g ccg tgc t ac at g cag ggc ccg t gg cac ggc ct c aag ct g ttc 1056
 Ala Leu Pro Cys Tyr Met Gl n Gy Pro Tr p His Gy Leu Lys Leu Phe
 340 345 350
 gcg at c gcg cac ttt acg tgc ggc gag gt g ct c gca acc at g ttc att 1104
 Ala Ile Ala His Phe Thr Cys Gy Gl u Val Leu Ala Thr Met Phe Ile
 355 360 365
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 Val Asn His Val Ile Gl u Gy Val Ser Tyr Ala Ser Lys Asp Ala Val
 370 375 380
 aag ggc acg at g gcg ccg ccg aag acg at g cac ggc gt g acg ccc at g 1200
 Lys Gy Thr Met Ala Pro Pro Lys Thr Met His Gy Val Thr Pro Met
 385 390 395 400
 aac aac acg cgc aag gag gt g gag gcg gag gcg t cc aag t ct ggc gcc 1248
 Asn Asn Thr Arg Lys Gl u Val Gl u Ala Gl u Ala Ser Lys Ser Gy Ala
 405 410 415
 gt g gt c aag t ca gt c ccg ct c gac gac t gg gcc gcc gt c cag t gc cag 1296
 Val Val Lys Ser Val Pro Leu Asp Asp Tr p Ala Ala Val Gl n Cys Gl n
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 acc t cg gcg aac t gg agc gt c ggc t cg t gg ttc t gg aac cac ttt t cc 1344
 Thr Ser Ala Asn Tr p Ser Val Gy Ser Tr p Phe Tr p Asn His Phe Ser
 435 440 445
 ggc ggt ct c aac cac cag att gag cac cac ct g ttc ccc ggg ct c agc 1392
 Gy Gy Leu Asn His Gl n Ile Gl u His His Leu Phe Pro Gy Leu Ser
 450 455 460
 cac gag acg t ac t ac cac at c cag gac gt c gt t cag t cc acc t gc gcc 1440
 His Gl u Thr Tyr Tyr His Ile Gl n Asp Val Val Gl n Ser Thr Cys Ala
 465 470 475 480
 gag t ac ggc gt c ccg t ac cag cac gag cct t cg ct c t gg acc gcg t ac 1488
 Gl u Tyr Gy Val Pro Tyr Gl n His Gl u Pro Ser Leu Tr p Thr Ala Tyr
 485 490 495
 t gg aag at g ct c gag cac ct c cgt cgg ct c ggc aat gag gag acc cac 1536
 Tr p Lys Met Leu Gl u His Leu Arg Arg Leu Gy Asn Gl u Gl u Thr His
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 Gl u Ser Tr p Gl n Arg Ala Ala
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<210> 80
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 <212> PRT
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 His Asn Lys Pro Asp Asp Ala Tr p Cys Ala Ile His Gy His Val Tyr
 20 25 30

PF58307.txt

Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
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 Leu Ala Ala Gly Lys Asp Ala Thr Val Leu Tyr Gu 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 G n Gly Gly Ala Asn Gu Lys Gu 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 Gu Arg Val Val Ala Arg Leu Lys Gu Arg Gly Lys Ala
 115 120 125
 Arg Arg Gly Gly Tyr Gu Leu Trp Ile Lys Ala 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 G n His Asp Gly Asn His Gly Ala Phe Ala G n 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 Gu Phe G n His Ala Leu Gly His His Pro Tyr
 210 215 220
 Thr Asn Leu Ile Gu Gu Gu Asn Gly Leu G n Lys Val Ser Gly Lys
 225 230 235 240
 Lys Met Asp Thr Lys Leu Ala Asp G n Gu Ser Asp Pro Asp Val Phe
 245 250 255
 Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His G n Lys Arg Trp
 260 265 270
 Tyr His Arg Phe G n His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
 275 280 285
 Thr Ile Asn Lys Val Val Thr G n Asp Val Gly Val Val Phe Arg Lys
 290 295 300
 Arg Leu Phe G n Ile Asp Ala Gu 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 G n Gly Pro Trp His Gly Leu Lys Leu Phe
 340 345 350
 Ala Ile Ala His Phe Thr Cys Gly Gu Val Leu Ala Thr Met Phe Ile
 355 360 365
 Val Asn His Val Ile Gu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
 370 375 380
 Lys Gly Thr Met Ala Pro Lys Thr Met His Gly Val Thr Pro Met
 385 390 395 400
 Asn Asn Thr Arg Lys Gu Val Gu Ala Gu Ala Ser Lys Ser Gly Ala
 405 410 415
 Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val G n Cys G n
 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 G n Ile Gu His His Leu Phe Pro Gly Leu Ser
 450 455 460
 His Gu Thr Tyr Tyr His Ile G n Asp Val Val G n Ser Thr Cys Ala
 465 470 475 480
 Gu Tyr Gly Val Pro Tyr G n His Gu Pro Ser Leu Trp Thr Ala Tyr
 485 490 495
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 Gu Ser Trp G n Arg Ala Ala

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 <212> DNA
 <213> Thal assi osi r a pseudonana

<220>
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 Ser Lys Pro Gln Gln Gln His Gu His Arg Thr Ile Ser Lys Ser Gu
 20 25 30
 ct c gcc caa cac aac acg ccc aaa t ca gca t gg t gt gcc gt c cac t cc 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 t cc cac t cc aac aac aaa caa cac gca cac 192
 Thr Pro Ala Thr Asp Pro Ser His Ser Asn Asn Lys Gln His Ala His
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 Tyr His Pro Arg Gly Val Pro Thr Ser Leu Ile Gln Lys Leu Gln Ile
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 Gly Val Met Gu Gu Gu Ala Phe Arg Asp Ser Phe Tyr Ser Trp Thr
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 Asp Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg Val Val Gu Arg Leu
 130 135 140
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 Gly Asn His Gly Ala Phe Ala Gln Asn Lys Leu Leu Asn Lys Leu Ala
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 325 330 335

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 Lys Val Ile Thr Met Gly Tyr Met Gly Leu Pro Ile Tyr Phe His
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 Gly Val Leu Arg Gly Val Gly Leu Phe Val Ile Gly His Leu Ala Cys
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 Gly Gu Leu Leu Ala Thr Met Phe Ile Val Asn His Val Ile Gu Gly
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 Val Ser Tyr Gly Thr Lys Asp Leu Val Gly Ala Ser His Gly Asp
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 Gu Lys Lys Ile Val Lys Pro Thr Thr Val Leu Gly Asp Thr Pro Met
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 Gu Lys Thr Arg Gu Gu Ala Leu Lys Ser Asn Ser Asn Asn Asn Lys
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 Tyr His Pro Arg Gly Val Pro Thr Ser Leu Ile Gl n Lys Leu Gl n Ile
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 Gly Val Met Gu Gu Ala Phe Arg Asp Ser Phe Tyr Ser Trp Thr
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 Sei te 107

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 Val Ser Tyr Gly Thr Lys Asp Leu Val Gly Ala Ser His Gly Asp
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 Gu Lys Thr Arg Gu Gu Ala Leu Lys Ser Asn Ser Asn Asn Asn Lys
 Lys Lys Gly Gu Lys Asn Ser Val Pro Ser Val Pro Phe Asn Asp Trp
 Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser Pro Gly Ser Trp
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aac Asn	gtt Val	cgg Arg	ccc Pro	acc Thr	cgt Arg	cca Al a	gct Al a	gga G y	ccc Pro	ccg Pro	ccg Pro	gcc Al a	acg Thr	tac Tyr	tac Tyr	192
gac Asp	tcc Ser	ctg Leu	gca Al a	gtg Val	tcg Ser	ggg G y	cag G n	ggc G y	aag Lys	gag G u	cgg Arg	ctg Leu	ttc Phe	acc Thr	acc Thr	240
gat Asp	gag G u	gtg Val	agg Arg	cgg Arg	cac His	atc Ile	ctc Leu	ccc Pro	acc Thr	gat Asp	ggc G y	tgg Trp	ctg Leu	acg Thr	tgc Cys	288
cac His	gaa G u	gga G y	gtc Val	tac Tyr	gat Asp	gtc Val	act Thr	gat Asp	ttc Phe	ctt Leu	gcc Al a	aag Lys	cac His	cct Pro	ggt G y	336
ggc G y	ggt G y	gtc Val	atc Ile	acg Thr	ctg Leu	ggc G y	ctt Leu	gga G y	agg Arg	gac Asp	tgc Cys	aca Thr	atc Ile	ctc Leu	atc Ile	384
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cgc Arg	att Ile	ggt G y	acg Thr	ctg Leu	cag G n	gac Asp	ccc Pro	aag Lys	acg Thr	ttc Tyr	tat Tyr	gct Al a	tgg Trp	gga G y	gag G u	480
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gtg Val	ctc Leu	acc Thr	ctc Leu	ttc Phe	ttc Phe	gtg Val	tcg Ser	tgg Trp	tac Tyr	atg Met	tgg Trp	gtg Trp	gcc Al a	cac His	aag Lys	624
tcc Ser	ttc Phe	ctc Leu	tgg Trp	gcc Al a	gcc Al a	gtc Val	tgg Trp	ggc G y	ttc Phe	gcc Al a	ggc G y	tcc Ser	cac His	gtc Val	ggg G y	672
ctg Leu	agc Ser	atc Ile	cag G n	cac His	gat Asp	ggy G y	aac Asn	cac His	ggc G y	cgc Al a	ttc Phe	agc Ser	cgc Arg	aac Asn	aca Thr	720
ctg Leu	gtg Val	aac Asn	cgc Arg	ctg Leu	gcg Al a	ggg G y	tgg Trp	ggc G y	atg Met	gac Asp	tig Leu	atc Ile	ggc G y	cgc Al a	tcg Ser	768
tcc Ser	acg Thr	gtg Val	tgg Trp	gag G u	tac Tyr	cag G n	cac His	gtc Val	atc Ile	ggc G y	cac His	cac His	cag G n	tac Tyr	acc Thr	816
aac Asn	ctc Leu	gtg Val	tcg Ser	gac Asp	acg Thr	cta Leu	ttc Phe	agt Ser	ctg Leu	cct Pro	gag G u	aac Asn	gat Asp	ccg Pro	gac Asp	864
gtc Val	ttc Phe	tcc Ser	agc Ser	tac Tyr	ccg Pro	ctg Leu	atg Met	cgc Arg	atg Met	cac His	ccg Pro	gat Asp	acg Thr	cgc Al a	tgg Trp	912
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ctg Leu	atg Met	aca Thr	atc Ile	agc Ser	aag Lys	gtg Val	ctg Leu	acc Thr	agc Ser	gat Asp	ttc Phe	gct Al a	gtc Val	tgc Cys	ctc Leu	1008
agc Ser	atg Met	aag Lys	aag Lys	ggg G y	tcc Ser	atc Ile	gac Asp	tgc Cys	tcc Ser	tcc Ser	agg Arg	ctc Leu	gtc Val	cca Pro	ctg Leu	1056
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cag G n	att Ile	gtg Val	tig Leu	cca Pro	tgc Cys	tac Tyr	ctc Leu	cac His	ggg G y	aca Thr	gct Al a	ggc Met	ctg G y	gcc Leu	gac Al a	1152
ctc Leu	ttc Phe	tct Ser	gtt Val	gct Al a	cac His	ctt Leu	gtg Val	tcg Ser	ggg G y	gag G u	tac Tyr	ctc Leu	cgc Al a	atc Ile	tgc Cys	1200

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				405					410						415		
	t t t	c a a	a c c	g c c	g c c	c g g	a g g	a c a	g a g	a t g	c t t	c a g	g c a	g c a	c a t	c a g	1296
	P h e	G l n	T h r	A l a	A l a	A r g	A r g	T h r	G u	M e t	L e u	G l n	A l a	A l a	H i s	G l n	
				420					425					430			
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	A l a	A l a	G l u	A l a	L y s	L y s	V a l	L y s	P r o	T h r	P r o	P r o	P r o	A s n	A s p	T r p	
			435					440					445				
	g c t	g t g	a c a	c a g	g t c	c a a	t g c	t g c	g t g	a a t	t g g	a g a	t c a	g g t	g g c	g t g	1392
	A l a	V a l	T h r	G l n	V a l	G l n	C y s	C y s	V a l	A s n	T r p	A r g	S e r	G l y	G l y	V a l	
		450					455					460					
	t t g	g c c	a a t	c a c	c t c	t c t	g g a	g g c	t t g	a a c	c a c	c a g	a t c	g a g	c a t	c a t	1440
	L e u	A l a	A s n	H i s	L e u	S e r	G l y	G l y	L e u	A s n	H i s	G l n	I l e	G l u	H i s	H i s	
	465					470					475					480	
	c t g	t t c	c c c	a g c	a t c	t c g	c a t	g c c	a a c	t a c	c c c	a c c	a t c	g c c	c c t	g t t	1488
	L e u	P h e	P r o	S e r	I l e	S e r	H i s	A l a	A s n	T y r	P r o	T h r	I l e	A l a	P r o	V a l	
				485					490						495		
	g t g	a a g	g a g	g t g	t g c	g a g	g a g	t a c	g g g	t t g	c c g	t a c	a a g	a a t	t a c	g t c	1536
	V a l	L y s	G l u	V a l	C y s	G l u	G l u	T y r	G l y	L e u	P r o	T y r	L y s	A s n	T y r	V a l	
			500					505					510				
	a c g	t t c	t g g	g a t	g c a	g t c	t g t	g g c	a t g	g t t	c a g	c a c	c t c	c g g	t t g	a t g	1584
	T h r	P h e	T r p	A s p	A l a	V a l	C y s	G l y	M e t	V a l	G l n	H i s	L e u	A r g	L e u	M e t	
			515				520						525				
	g g t	g c t	c c a	c c g	g t g	c c a	a c g	a a c	g g g	g a c	a a a	a a g	t c a	t a a			1626
	G l y	A l a	P r o	P r o	V a l	P r o	T h r	A s n	G l y	A s p	L y s	L y s	S e r				
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	G l n	P r o	C y s	G l u	A s n	G l y	T h r	V a l	G l u	L y s	A r g	G l u	A s n	A s p	T h r
			35					40					45		
	A s n	V a l	A r g	P r o	T h r	A r g	P r o	A l a	G l y	P r o	P r o	P r o	A l a	T h r	T y r
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	H i s	G l u	G l y	V a l	T y r	A s p	V a l	T h r	A s p	P h e	L e u	A l a	L y s	H i s	P r o
				100					105					110	
	G l y	G l y	V a l	I l e	T h r	L e u	G l y	L e u	G l y	A r g	A s p	C y s	T h r	I l e	L e u
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	G l u	S e r	T y r	H i s	P r o	A l a	G l y	A r g	P r o	A s p	L y s	V a l	M e t	G l u	L y s
			130				135					140			
	A r g	I l e	G l y	T h r	L e u	G l n	A s p	P r o	L y s	T h r	P h e	T y r	A l a	T r p	G l y
		145				150						155			160
	S e r	A s p	P h e	T y r	P r o	G l u	L e u	L y s	A r g	A r g	A l a	L e u	A l a	A r g	L e u
				165					170					175	
	G l u	A l a	G l y	G l n	A l a	A r g	A r g	G l y	G l y	L e u	G l y	V a l	L y s	A l a	L e u
			180					185					190		
	V a l	L e u	T h r	L e u	P h e	P h e	V a l	S e r	T r p	T y r	M e t	T r p	V a l	A l a	H i s
			195					200					205		
	S e r	P h e	L e u	T r p	A l a	A l a	V a l	T r p	G l y	P h e	A l a	G l y	S e r	H i s	V a l
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		225				230						235			240
	L e u	V a l	A s n	A r g	L e u	A l a	G l y	T r p	G l y	M e t	A s p	L e u	I l e	G l y	A l a
				245						250				255	
	S e r	T h r	V a l	T r p	G l u	T y r	G l n	H i s	V a l	I l e	G l y	H i s	H i s	G l n	T y r

Sei t e 110

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 Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu
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 Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu
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 G n Ile Val Leu Pro Cys Tyr Leu His Gy Thr Ala Met Gy Leu Ala
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 Phe Ile Ile Asn His Ile Ser Gu Ser Cys Gy Glu Phe Met Asn Thr Ser
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 Asp Ile Thr Lys Phe Ser Lys Val His Pro Gy Gy Asp Ile Ile Met
 35 40 45
 ct g gcc gct ggc aag gag gcc acc at c ct g t t c gag acc t ac cac at c 192
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 Lys Gy Val Pro Asp Ala Val Leu Arg Lys Tyr 75 Lys Val Gy Lys 80
 65 70 75
 ccc cag ggc aag aag ggc gaa acg agc cac at g ccc acc ggg ct c gac 288
 Pro G n Gy Lys Lys Gy Gu Thr Ser His Met Pro Thr Gy Leu Asp
 85 90 95
 t cg gcc t cc t ac t ac t cg t gg gac agc gag t t t t ac agg gt g ct c cgc 336
 Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Gu Phe Tyr Arg Val Leu Arg
 100 105 110
 gag cgc gt c gcc aag aag ct g gcc gag ccc ggc ct c at g cag cgc gcg 384
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Gu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Gu	Pro	Gly	Leu	Met	Gln	Arg	Ala		
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		
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		
gt a	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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
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		
tct	gtg	aac	tgg	gct	gtc	ggg	tcg	ttt	tgg	aac	cac	ttt	tcg	ggc			1344
Ser	Val	Asn	Trp	Ala	Val	Gly	Ser	Trp	Phe	Trp	Asn	His	Phe	Ser	Gly		
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		
acg	gtc	aac	gtc	tac	atc	tgc	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		

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Tyr	G y	Val	Pro	Tyr	G n	Al a	G u	I l e	Ser	Leu	Phe	Ser	Al a	Tyr	Phe	
				485					490					495		
aag	at g	ct g	t cg	cac	ct c	cgc	acg	ct c	ggc	aac	gag	gac	ct c	acg	gcc	1536
Lys	Mæt	Leu	Ser	Hi s	Leu	Arg	Thr	Leu	G y	Asn	G u	Asp	Leu	Thr	Al a	
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t gg	t cc	acg	t ga													1548
Tr p	Ser	Thr														
		515														

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 <212> PRT
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			20					25					30			
Asp	I l e	Thr	Lys	Phe	Ser	Lys	Val	Hi s	Pro	G y	G y	Asp	I l e	I l e	Mæt	
		35					40					45				
Leu	Al a	Al a	G y	Lys	G u	Al a	Thr	I l e	Leu	Phe	G u	Thr	Tyr	Hi s	I l e	
	50					55					60					
Lys	G y	Val	Pro	Asp	Al a	Val	Leu	Arg	Lys	Tyr	Lys	Val	G y	Lys	Leu	
65					70					75				80		
Pro	G n	G y	Lys	Lys	G y	G u	Thr	Ser	Hi s	Mæt	Pro	Thr	G y	Leu	Asp	
				85					90					95		
Ser	Al a	Ser	Tyr	Tyr	Ser	Tr p	Asp	Ser	G u	Phe	Tyr	Arg	Val	Leu	Arg	
			100					105					110			
G u	Arg	Val	Al a	Lys	Lys	Leu	Al a	G u	Pro	G y	Leu	Mæt	G n	Arg	Al a	
		115					120					125				
Arg	Mæt	G u	Leu	Tr p	Al a	Lys	Al a	I l e	Phe	Leu	Leu	Al a	G y	Phe	Tr p	
	130					135					140					
G y	Ser	Leu	Tyr	Al a	Mæt	Cys	Val	Leu	Asp	Pro	Hi s	G y	G y	Al a	Mæt	
145					150				155					160		
Val	Al a	Al a	Val	Thr	Leu	G y	Val	Phe	Al a	Al a	Phe	Val	G y	Thr	Cys	
				165					170					175		
I l e	G n	Hi s	Asp	G y	Ser	Hi s	G y	Al a	Phe	Ser	Lys	Ser	Arg	Phe	Mæt	
			180					185					190			
Asn	Lys	Al a	Al a	G y	Tr p	Thr	Leu	Asp	Mæt	I l e	G y	Al a	Ser	Al a	Mæt	
		195					200					205				
Thr	Tr p	G u	Mæt	G n	Hi s	Val	Leu	G y	Hi s	Hi s	Pro	Tyr	Thr	Asn	Leu	
	210					215					220					
I l e	G u	Mæt	G u	Asn	G y	Leu	Al a	Lys	Val	Lys	G y	Al a	Asp	Val	Asp	
225					230					235					240	
Pro	Lys	Lys	Val	Asp	G n	G u	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr	
				245					250					255		
Pro	Mæt	Leu	Arg	Leu	Hi s	Pro	Tr p	Hi s	Arg	G n	Arg	Phe	Tyr	Hi s	Lys	
			260					265					270			
Phe	G n	Hi s	Leu	Tyr	Al a	Pro	Phe	I l e	Phe	G y	Ser	Mæt	Thr	I l e	Asn	
		275					280					285				
Lys	Val	I l e	Ser	G n	Asp	Val	G y	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe	
	290					295					300					
G n	I l e	Asp	Al a	Asn	Cys	Arg	Tyr	G y	Ser	Pro	Tr p	Tyr	Val	Al a	Arg	
305					310					315					320	
Phe	Tr p	I l e	Mæt	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Mæt	Val	Al a	Leu	Pro	
				325					330					335		
Mæt	Tyr	Mæt	G n	G y	Pro	Al a	G n	G y	Leu	Lys	Leu	Phe	Phe	Mæt	Al a	
			340					345					350			
Hi s	Phe	Thr	Cys	G y	G u	Val	Leu	Al a	Thr	Mæt	Phe	I l e	Val	Asn	Hi s	
		355					360					365				
I l e	I l e	G u	G y	Val	Ser	Tyr	Al a	Ser	Lys	Asp	Al a	Val	Lys	G y	Val	
	370					375					380					
Mæt	Al a	Pro	Pro	Arg	Thr	Val	Hi s	G y	Val	Thr	Pro	Mæt	G n	Val	Thr	
385					390					395					400	
G n	Lys	Al a	Leu	Ser	Al a	Al a	G u	Ser	Al a	Lys	Ser	Asp	Al a	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
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 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 Gu His His Cys Phe Pro Gln Asn Pro His
 450 455 460
 Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Gu Thr Cys Gu Gu
 465 470 475 480
 Tyr Gly Val Pro Tyr Gln Ala Gu Ile Ser Leu Phe Ser Ala Tyr Phe
 485 490 495
 Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Gu Asp Leu Thr Ala
 500 505 510
 Trp Ser Thr
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 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 Gu Ala Thr Ile Leu Phe Gu Thr Tyr His Ile
 50 55 60
 aag ggc gtt ccg gac gcg gtg ctg cgc aag tac aag gtc ggc aag ctg 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 ggc ctg gac 288
 Pro Gln Gly Lys Lys Gly Gu 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 ctg cgc 336
 Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Gu Phe Tyr Arg Val Leu Arg
 100 105 110
 gag cgc gtc gcc aag aag ctg gcc gag ccc ggc ctg atg cag cgc gcg 384
 Gu Arg Val Ala Lys Lys Leu Ala Gu Pro Gly Leu Met Gln Arg Ala
 115 120 125
 cgc atg gag ctg tgg gcc aag gcg atc ttc ctg ctg gca ggt ttc tgg 432
 Arg Met Gu Leu Trp Ala Lys Ala Ile Phe 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 ctg gcc 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 gcc 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 gcc tgg acc ctg 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 ctg 672
 Thr Trp Gu 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

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I l e	G u	M e t	G u	A s n	G y	L e u	A l a	L y s	V a l	L y s	G y	A l a	A s p	V a l	A s p	
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c c g	a a g	a a g	g t c	g a c	c a g	g a g	a g c	g a c	c c g	g a c	g t c	t t c	a g t	a c g	t a c	768
P r o	L y s	L y s	V a l	A s p	G n	G u	S e r	A s p	P r o	A s p	V a l	P h e	S e r	T h r	T y r	
				245					250					255		
c c g	a t g	c t t	c g c	c t g	c a c	c c g	t g g	c a c	c g c	c a g	c g g	t t t	t a c	c a c	a a g	816
P r o	M e t	L e u	A r g	L e u	H i s	P r o	T r p	H i s	A r g	G n	A r g	P h e	T y r	H i s	L y s	
			260					265					270			
t t c	c a g	c a c	c t g	t a c	g c c	c c g	t t t	a t c	t t t	g g g	t t t	a t g	a c g	a t t	a a c	864
P h e	G n	H i s	L e u	T y r	A l a	P r o	P h e	I l e	P h e	G y	P h e	M e t	T h r	I l e	A s n	
			275				280					285				
a a g	g t g	a t t	t c c	c a g	g a t	g t c	g g g	g t t	g t g	c t g	c g c	a a g	c g c	c t g	t t c	912
L y s	V a l	I l e	S e r	G n	A s p	V a l	G y	V a l	V a l	L e u	A r g	L y s	A r g	L e u	P h e	
			290			295					300					
c a g	a t c	g a c	g c c	a a c	t g c	c g g	t a t	g g c	a g c	c c c	t g g	t a c	g t g	g c c	c g c	960
G n	I l e	A s p	A l a	A s n	C y s	A r g	T y r	G y	S e r	P r o	T r p	T y r	V a l	A l a	A r g	
305				310					315						320	
t t c	t g g	a t c	a t g	a a g	c t c	c t c	a c c	a c g	c t c	a t g	g t g	g c g	c t t	c c c		1008
P h e	T r p	I l e	M e t	L y s	L e u	L e u	T h r	T h r	L e u	T y r	M e t	V a l	A l a	L e u	P r o	
				325				330						335		
a t g	t a c	a t g	c a g	g g g	c c t	g c t	c a g	g g c	t t g	a a g	c t t	t t c	t t c	a t g	g c c	1056
M e t	T y r	M e t	G n	G y	P r o	A l a	G n	G y	L e u	L y s	L e u	P h e	P h e	M e t	A l a	
			340					345					350			
c a c	t t c	a c c	t g c	g g a	g a g	g t c	c t c	g c c	a c c	a t g	t t t	a t t	g t c	a a c	c a c	1104
H i s	P h e	T h r	C y s	G y	G u	V a l	L e u	A l a	T h r	M e t	P h e	I l e	V a l	A s n	H i s	
			355				360					365				
a t c	a t c	g a g	g g c	g t c	a g c	t a c	g c t	t c c	a a g	g a c	g c g	g t c	a a g	g g c	g t c	1152
I l e	I l e	G u	G y	V a l	S e r	T y r	A l a	S e r	L y s	A s p	A l a	V a l	L y s	G y	V a l	
			370			375					380					
a t g	g c t	c c g	c c g	c g c	a c t	g t g	c a c	g g t	g t c	a c c	c c g	a t g	c a g	g t g	a c g	1200
M e t	A l a	P r o	P r o	A r g	T h r	V a l	H i s	G y	V a l	T h r	P r o	M e t	G n	V a l	T h r	
				390						395					400	
c a a	a a g	g c g	c t c	a g t	g c g	g c c	g a g	t c g	a c c	a a g	t c g	g a c	g c c	g a c	a a g	1248
G n	L y s	A l a	L e u	S e r	A l a	A l a	G u	S e r	T h r	L y s	S e r	A s p	A l a	A s p	L y s	
				405					410					415		
a c g	a c c	a t g	a t c	c c c	c t c	a a c	g a c	t g g	g c c	g c t	g t g	c a g	t g c	c a g	a c c	1296
T h r	T h r	M e t	I l e	P r o	L e u	A s n	A s p	T r p	A l a	A l a	V a l	G n	C y s	G n	T h r	
			420					425					430			
t c t	g t g	a a c	t g g	g c t	g t c	g g g	t c g	t g g	t t t	t g g	a a c	c a c	t t t	t c g	g g c	1344
S e r	V a l	A s n	T r p	A l a	V a l	G y	S e r	T r p	P h e	T r p	A s n	H i s	P h e	S e r	G y	
			435				440						445			
g g c	c t c	a a c	c a c	c a g	a t t	g a g	c a c	c a c	t g c	t t c	c c c	c a a	a a c	c c c	c a c	1392
G y	L e u	A s n	H i s	G n	I l e	G u	H i s	H i s	C y s	P h e	P r o	G n	A s n	P r o	H i s	
				450		455					460					
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T h r	V a l	A s n	V a l	T y r	I l e	S e r	G y	I l e	V a l	L y s	G u	T h r	C y s	G u	G u	
				465		470				475					480	
t a c	g g c	g t g	c c g	t a c	c a g	g c t	g a g	a t c	a g c	c t c	t t c	t c t	g c c	t a t	t t c	1488
T y r	G y	V a l	P r o	T y r	G n	A l a	G u	I l e	S e r	L e u	P h e	S e r	A l a	T y r	P h e	
				485					490					495		
a a g	a t g	c t g	t c g	c a c	c t c	c g c	a c g	c t c	g g c	a a c	g a g	g a c	c t c	a c g	g c c	1536
L y s	M e t	L e u	S e r	H i s	L e u	A r g	T h r	L e u	G y	A s n	G u	A s p	L e u	T h r	A l a	
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T r p	S e r	T h r														
		515														

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 1 5 10 15
 H i s A s n M e t P r o A s p A s p A l a T r p C y s A l a I l e H i s G l y T h r V a l T y r
 20 25 30
 A s p I l e T h r L y s P h e S e r L y s V a l H i s P r o G l y G l y A s p I l e I l e M e t
 S e i t e 115

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Leu Ala Ala Gly Lys Gu Ala Thr Ile Leu Phe Gu Thr Tyr His Ile
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 65 70 75 80
 Pro Gn Gly Lys Lys Gly Gu Thr Ser His Met Pro Thr Gly Leu Asp
 85 90 95
 Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Gu Phe Tyr Arg Val Leu Arg
 100 110
 Gu Arg Val Ala Lys Lys Leu Ala Gu Pro Gly Leu Met Gn Arg Ala
 115 120 125
 Arg Met Gu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
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 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 Gn His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
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 Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
 195 200 205
 Thr Trp Gu Met Gn His Val Leu Gly His His Pro Tyr Thr Asn Leu
 210 215 220
 Ile Gu Met Gu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
 225 230 235 240
 Pro Lys Lys Val Asp Gn Gu Ser Asp Pro Asp Val Phe Ser Thr Tyr
 245 250 255
 Pro Met Leu Arg Leu His Pro Trp His Arg Gn Arg Phe Tyr His Lys
 260 265 270
 Phe Gn His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
 275 280 285
 Lys Val Ile Ser Gn Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
 290 295 300
 Gn Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
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 Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
 325 330 335
 Met Tyr Met Gn Gly Pro Ala Gn Gly Leu Lys Leu Phe Phe Met Ala
 340 345 350
 His Phe Thr Cys Gly Gu Val Leu Ala Thr Met Phe Ile Val Asn His
 355 360 365
 Ile Ile Gu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
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 Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gn Val Thr
 385 390 395 400
 Gn Lys Ala Leu Ser Ala Ala Gu Ser Thr Lys Ser Asp Ala Asp Lys
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 Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gn Cys Gn Thr
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 Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
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 Gly Leu Asn His Gn Ile Gu His His Cys Phe Pro Gn Asn Pro His
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 465 470 475 480
 Tyr Gly Val Pro Tyr Gn Ala Gu Ile Ser Leu Phe Ser Ala Tyr Phe
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cac	aac	at g	ccg	gac	gac	gcc	t gg	t gc	gcg	at c	cac	ggc	acc	gt g	t ac	96
His	Asn	Met	Pro	Asp	Asp	Ala	Trp	Cys	Ala	Ile	His	Gly	Thr	Val	Tyr	
		20						25				30				
gac	at c	acc	aag	t t c	agc	aag	gt g	cac	ccc	ggc	ggg	gac	at c	at c	at g	144
Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met	
	35						40					45				
ct g	gcc	gct	ggc	aag	gag	gcc	acc	at c	ct g	t t c	gag	acg	t ac	cac	at c	192
Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile	
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aag	ggc	gt c	ccg	gac	gcg	gt g	ct g	cgc	aag	t ac	aag	gt c	ggc	aag	ct c	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	at g	ccc	acc	ggg	ct c	gac	288
Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp	
			85						90					95		
t cg	gcc	t cc	t ac	t ac	t cg	t gg	gac	agc	gag	t t t	t ac	agg	gt g	ct c	cgc	336
Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg	
			100					105					110			
gag	cgc	gt c	gcc	aag	aag	ct g	gcc	gag	ccc	ggc	ct c	at g	cag	cgc	gcg	384
Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala	
		115					120					125				
cgc	at g	gag	ct c	t gg	gcc	aag	gcg	at c	t t c	ct c	ct g	gca	ggg	t t c	t gg	432
Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp	
	130				135						140					
ggc	t cc	ct t	t ac	gcc	at g	t gc	gt g	ct a	gac	ccg	cac	ggc	ggg	gcc	at g	480
Gly	Ser	Leu	Tyr	Ala	Met	Cys	Val	Leu	Asp	Pro	His	Gly	Gly	Ala	Met	
	145				150					155					160	
gt a	gcc	gcc	gt t	acg	ct c	ggc	gt g	t t c	gct	gcc	t t t	gt c	gga	act	t gc	528
Val	Ala	Ala	Val	Thr	Leu	Gly	Val	Phe	Ala	Ala	Phe	Val	Gly	Thr	Cys	
			165						170					175		
at c	cag	cac	gac	ggc	agc	cac	ggc	gcc	t t c	t cc	aag	t cg	cga	t t c	at g	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	t gg	acc	ct c	gac	at g	at c	ggc	gcg	agc	gcg	at g	624
Asn	Lys	Ala	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met	
		195					200					205				
acc	t gg	gag	at g	cag	cac	gt t	ct t	ggc	cac	cac	ccg	t ac	acc	aac	ct c	672
Thr	Trp	Glu	Met	Gln	His	Val	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Leu	
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at c	gag	at g	gag	aac	ggg	t t g	gcc	aag	gt c	aag	ggc	gcc	gac	gt c	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	gt c	gac	cag	gag	agc	gac	ccg	gac	gt c	t t c	agt	acg	t ac	768
Pro	Lys	Lys	Val	Asp	Gln	Glu	Ser	Asp	Pro	Asp	Val	Phe	Ser	Thr	Tyr	
			245						250					255		
ccg	at g	ct t	cgc	ct g	cac	ccg	t gg	cac	cgc	cag	ccg	t t t	t ac	cac	aag	816
Pro	Met	Leu	Arg	Leu	His	Pro	Trp	His	Arg	Gln	Arg	Phe	Tyr	His	Lys	
			260					265								
t t c	cag	cac	ct g	t ac	gcc	ccg	t t t	at c	t t t	ggg	t t t	at g	acg	at t	aac	864
Phe	Gln	His	Leu	Tyr	Ala	Pro	Phe	Ile	Phe	Gly	Phe	Met	Thr	Ile	Asn	
			275				280					285				
aag	gt g	at t	t cc	cag	gat	gt c	ggg	gt t	gt g	ct g	cgc	aag	cgc	ct g	t t c	912
Lys	Val	Ile	Ser	Gln	Asp	Val	Gly	Val	Val	Leu	Arg	Lys	Arg	Leu	Phe	
	290					295					300					
cag	at c	gac	gcc	aac	t gc	cgg	t at	ggc	agc	ccc	t gg	t ac	gt g	gcc	cgc	960
Gln	Ile	Asp	Ala	Asn	Cys	Arg	Tyr	Gly	Ser	Pro	Trp	Tyr	Val	Ala	Arg	
			305		310					315					320	
t t c	t gg	at c	at g	aag	ct c	ct c	acc	acg	ct c	t ac	acg	gt g	gcg	ct t	ccc	1008
Phe	Trp	Ile	Met	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Thr	Val	Ala	Leu	Pro	
			325						330					335		
at g	t ac	at g	cag	ggg	cct	gct	cag	ggc	t t g	aag	ct t	t t c	t t c	at g	gcc	1056

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Met	Tyr	Met	Gln	Gly	Pro	Ala	Gln	Gly	Leu	Lys	Leu	Phe	Phe	Met	Ala	
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cac	t t c	acc	t g c	g g a	g a g	g t c	c t c	g c c	acc	a t g	t t t	a t t	g t c	a a c	c a c	1104
His	Phe	Thr	Cys	Gly	Glu	Val	Leu	Ala	Thr	Met	Phe	Ile	Val	Asn	His	
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at c	at c	g a g	g g c	g t c	a g c	t a c	g c t	t c c	a a g	g a c	g c g	g t c	a a g	g g c	g t c	1152
Ile	Ile	Glu	Gly	Val	Ser	Tyr	Ala	Ser	Lys	Asp	Ala	Val	Lys	Gly	Val	
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at g	g c t	c c g	c c g	c g c	a c t	g t g	c a c	g g t	g t c	acc	c c g	a t g	c a g	g t g	a c g	1200
Met	Ala	Pro	Pro	Arg	Thr	Val	His	Gly	Val	Thr	Pro	Met	Gln	Val	Thr	
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caa	a a g	g c g	c t c	a g t	g c g	g c c	g a g	t c g	acc	a a g	t c g	g a c	g c c	g a c	a a g	1248
Gln	Lys	Ala	Leu	Ser	Ala	Ala	Glu	Ser	Thr	Lys	Ser	Asp	Ala	Asp	Lys	
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acg	acc	a t g	a t c	c c c	c t c	a a c	g a c	t g g	g c c	g c t	g t g	c a g	t g c	c a g	acc	1296
Thr	Thr	Met	Ile	Pro	Leu	Asn	Asp	Trp	Ala	Ala	Val	Gln	Cys	Gln	Thr	
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t c t	g t g	a a c	t g g	g c t	g t c	g g g	t c g	t g g	t t t	t g g	a a c	c a c	t t t	t c g	g g c	1344
Ser	Val	Asn	Trp	Ala	Val	Gly	Ser	Trp	Phe	Trp	Asn	His	Phe	Ser	Gly	
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g g c	c t c	a a c	c a c	c a g	a t t	g a g	c a c	c a c	t g c	t t c	c c c	c a a	a a c	c c c	c a c	1392
Gly	Leu	Asn	His	Gln	Ile	Glu	His	His	Cys	Phe	Pro	Gln	Asn	Pro	His	
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acg	g t c	a a c	g t c	t a c	a t c	t c g	g g c	a t c	g t c	a a g	g a g	acc	t g c	g a a	g a a	1440
Thr	Val	Asn	Val	Tyr	Ile	Ser	Gly	Ile	Val	Lys	Glu	Thr	Cys	Glu	Glu	
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t a c	g g c	g t g	c c g	t a c	c a g	g c t	g a g	a t c	a g c	c t c	t t c	t c t	g c c	t a t	t t c	1488
Tyr	Gly	Val	Pro	Tyr	Gln	Ala	Glu	Ile	Ser	Leu	Phe	Ser	Ala	Tyr	Phe	
				485					490					495		
a a g	a t g	c t g	t c g	c a c	c t c	c g c	a c g	c t c	g g c	a a c	g a g	g a c	c t c	a c g	g c c	1536
Lys	Met	Leu	Ser	His	Leu	Arg	Thr	Leu	Gly	Asn	Glu	Asp	Leu	Thr	Ala	
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agg	t c c	a c g	t g a													1548
Arg	Ser	Thr														
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Asp	Ile	Thr	Lys	Phe	Ser	Lys	Val	His	Pro	Gly	Gly	Asp	Ile	Ile	Met	
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Leu	Ala	Ala	Gly	Lys	Glu	Ala	Thr	Ile	Leu	Phe	Glu	Thr	Tyr	His	Ile	
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Pro	Gln	Gly	Lys	Lys	Gly	Glu	Thr	Ser	His	Met	Pro	Thr	Gly	Leu	Asp	
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Ser	Ala	Ser	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg		
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Glu	Arg	Val	Ala	Lys	Lys	Leu	Ala	Glu	Pro	Gly	Leu	Met	Gln	Arg	Ala	
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Arg	Met	Glu	Leu	Trp	Ala	Lys	Ala	Ile	Phe	Leu	Leu	Ala	Gly	Phe	Trp	
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Asn	Lys	Ala	Ala	Gly	Trp	Thr	Leu	Asp	Met	Ile	Gly	Ala	Ser	Ala	Met	
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 Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
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 Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
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 Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
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 Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
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 Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
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 Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
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 Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Thr Val Ala Leu Pro
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 Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
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 His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
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 Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
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 Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
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 Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
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 Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
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 Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
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 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
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 His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
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 gac at c acc aag t t c agc aag gt g cac ccc ggc ggg gac at c at c at g 144
 Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
 35 40 45
 ct g gcc gct ggc aag gag gcc acc at c ct g t t c gag acc t ac cac at c 192
 Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
 50 55 60
 aag ggc gt c ccg gac gcg gt g ct g cgc aag t ac aag gt c ggc aag ct c 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 at g ccc acc ggg ct c gac 288
 Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp

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Ser	Ala	Ser	Tyr	Tyr	Ser	Trp	Asp	Ser	Glu	Phe	Tyr	Arg	Val	Leu	Arg	
			100					105				110				
g a g	c g c	g t c	g c c	a a g	a a g	c t g	g c c	g a g	c c c	g g c	c t c	a t g	c a g	c g c	c g c	384
G u	A r g	V a l	A l a	L y s	L y s	L e u	A l a	G u	P r o	G l y	L e u	M e t	G l n	A r g	A l a	
		115					120					125				
c g c	a t g	g a g	c t c	t g g	g c c	a a g	g c g	a t c	t t c	c t c	c t g	g c a	g g t	t t c	t g g	432
A r g	M e t	G l u	L e u	T r p	A l a	L y s	A l a	l l e	P h e	L e u	L e u	A l a	G l y	P h e	T r p	
	130					135					140					
g g c	t c c	c t t	t a c	g c c	a t g	t g c	g t g	c t a	g a c	c c g	c a c	g g c	g g t	g c c	a t g	480
G l y	S e r	L e u	T y r	A l a	M e t	C y s	V a l	L e u	A s p	P r o	H i s	G l y	G l y	A l a	M e t	
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g t a	g c c	g c c	g t t	a c g	c t c	g g c	g t g	t t c	g c t	g c c	t t t	g t c	g g a	a c t	t g c	528
V a l	A l a	A l a	V a l	T h r	L e u	G l y	V a l	P h e	A l a	A l a	P h e	V a l	G l y	T h r	C y s	
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a t c	c a g	c a c	g a c	g g c	g c c	c a c	g g c	g c c	t t c	t c c	a a g	t c g	c g a	t t c	a t g	576
l l e	G l n	H i s	A s p	G l y	S e r	H i s	G l y	A l a	P h e	S e r	L y s	S e r	A r g	P h e	M e t	
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a a c	a a g	g c g	g c g	g g c	t g g	a c c	c t c	g a c	a t g	a t c	g g c	g c g	g c g	g c g	a t g	624
A s n	L y s	A l a	A l a	G l y	T r p	T h r	L e u	A s p	M e t	l l e	G l y	A l a	S e r	A l a	M e t	
		195					200				205					
a c c	t g g	g a g	a t g	c a g	c a c	g t t	c t t	g g t	c a c	c a c	c c g	t a c	a c c	a a c	c t c	672
T h r	T r p	G l u	M e t	G l n	H i s	V a l	L e u	G l y	H i s	H i s	P r o	T y r	T h r	A s n	L e u	
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a t c	g a g	a t g	g a g	a a c	g g t	t t g	g c c	a a g	g t c	a a g	g g c	g c c	g a c	g t c	g a c	720
l l e	G l u	M e t	G l u	A s n	G l y	L e u	A l a	L y s	V a l	L y s	G l y	A l a	A s p	V a l	A s p	
	225				230					235					240	
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P r o	L y s	L y s	V a l	A s p	G l n	G l u	S e r	A s p	P r o	A s p	V a l	P h e	S e r	T h r	T y r	
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c c g	a t g	c t t	c g c	c t g	c a c	c c g	t g g	c a c	c g c	c a g	c g g	t t t	t a c	c a c	a a g	816
P r o	M e t	L e u	A r g	L e u	H i s	P r o	T r p	H i s	A r g	G l n	A r g	P h e	T y r	H i s	L y s	
			260				265						270			
t t c	c a g	c a c	c t g	t a c	g c c	c c g	c t t	a t c	t t t	g g g	t t t	a t g	a c g	a t t	a a c	864
P h e	G l n	H i s	L e u	T y r	A l a	P r o	L e u	l l e	P h e	G l y	P h e	M e t	T h r	l l e	A s n	
		275					280					285				
a a g	g t g	a t t	t c c	c a g	g a t	g t c	g g g	g t t	g t g	c t g	c g c	a a g	c g c	c t g	t t c	912
L y s	V a l	l l e	S e r	G l n	A s p	V a l	G l y	V a l	V a l	L e u	A r g	L y s	A r g	L e u	P h e	
	290				295						300					
c a g	a t c	g a c	g c c	a a c	t g c	c g g	t a t	g g c	g c c	c c c	t g g	a a c	g t g	g c c	c g c	960
G l n	l l e	A s p	A l a	A s n	C y s	A r g	T y r	G l y	S e r	P r o	T r p	A s n	V a l	A l a	A r g	
	305				310					315					320	
t t c	t g g	a t c	a t g	a a g	c t c	c t c	a c c	a c g	c t c	t a c	a t g	g t g	g c g	c t t	c c c	1008
P h e	T r p	l l e	M e t	L y s	L e u	L e u	T h r	T h r	L e u	T y r	M e t	V a l	A l a	L e u	P r o	
			325					330						335		
a t g	t a c	a t g	c a g	g g g	c c t	g c t	c a g	g g c	t t g	a a g	c t t	t t c	t t c	a t g	g c c	1056
M e t	T y r	M e t	G l n	G l y	P r o	A l a	G l n	G l y	L e u	L y s	L e u	P h e	P h e	M e t	A l a	
		340					345						350			
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H i s	P h e	T h r	C y s	G l y	G l u	V a l	L e u	A l a	T h r	M e t	P h e	l l e	V a l	A s n	H i s	
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a t c	a t c	g a g	g g c	g t c	g c c	t a c	g c t	t c c	a a g	g a c	g c g	g t c	a a g	g g c	g t c	1152
l l e	l l e	G l u	G l y	V a l	S e r	T y r	A l a	S e r	L y s	A s p	A l a	V a l	L y s	G l y	V a l	
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M e t	A l a	P r o	P r o	A r g	T h r	V a l	H i s	G l y	V a l	T h r	P r o	M e t	G l n	V a l	T h r	
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G l n	L y s	A l a	L e u	S e r	A l a	A l a	G l u	S e r	T h r	L y s	S e r	A s p	A l a	A s p	L y s	
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T h r	T h r	M e t	l l e	P r o	L e u	A s n	A s p	T r p	A l a	A l a	V a l	G l n	C y s	G l n	T h r	
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S e r	V a l	A s n	T r p	A l a	V a l	G l y	S e r	T r p	P h e	T r p	A s n	H i s	P h e	S e r	G l y	
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Thr	Val	Asn	Val	Tyr	I l e	Ser	G y	I l e	Val	Lys	G u	Thr	C y s	G u	G u		
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Tyr	G y	Val	Pro	Tyr	G n	Al a	G u	I l e	Ser	Leu	P h e	Ser	Al a	Tyr	P h e		
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Tr p	Ser	Thr															
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Lys	Val	I l e	Ser	G n	Asp	Val	G y	Val	Val	Leu	Arg	Lys	Arg	Leu	P h e		
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G n	I l e	Asp	Al a	Asn	C y s	Arg	Tyr	G y	Ser	Pro	Tr p	Asn	Val	Al a	Arg		
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P h e	Tr p	I l e	Met	Lys	Leu	Leu	Thr	Thr	Leu	Tyr	Met	Val	Al a	Leu	Pro		
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Met	Tyr	Met	G n	G y	Pro	Al a	G n	G y	Leu	Lys	Leu	P h e	P h e	Met	Al a		
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Hi s	P h e	Thr	C y s	G y	G u	Val	Leu	Al a	Thr	Met	P h e	I l e	Val	Asn	Hi s		
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I l e	I l e	G u	G y	Val	Ser	Tyr	Al a	Ser	Lys	Asp	Al a	Val	Lys	G y	Val		

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 20 25 30
 xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
 35 40 45
 Val Arg xaa His Asn xaa Pro xaa Asp Ala Trp Cys Ala xaa His xaa
 50 55 60
 xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
 65 70 75 80
 xaa Val Tyr Asp xaa Thr xaa Phe xaa xaa xaa His Pro Gly Gly Asp
 85 90 95
 xaa Ile xaa Leu Ala xaa Gly Lys xaa Ala Thr xaa Leu xaa Gu Thr
 100 105 110
 Tyr His xaa xaa Gly Val xaa Asp xaa Val xaa xaa Lys Tyr xaa xaa
 115 120 125
 Gly xaa Leu xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa
 130 135 140
 xaa xaa xaa xaa xaa xaa xaa xaa xaa xaa Tyr xaa Trp xaa xaa Ser
 145 150 155 160
 xaa Phe Tyr xaa Val xaa xaa xaa Arg Val xaa xaa xaa Leu xaa Gu
 165 170 175
 xaa Gly xaa xaa xaa Arg xaa xaa xaa Gu Leu Trp xaa Lys Ala xaa
 180 185 190
 xaa Leu Leu xaa Gly Phe Trp xaa Ser Leu Tyr xaa Met xaa xaa xaa
 195 200 205
 xaa xaa xaa Asp xaa xaa xaa xaa Ala xaa xaa xaa Ala xaa xaa xaa
 210 215 220
 Gly xaa Phe Ala Ala Phe Val Gly Thr Cys Ile Gn His Asp Gly xaa
 225 230 235 240
 His Gly Ala Phe xaa xaa xaa xaa xaa Asn Lys xaa Ala Gly Trp
 245 250 255
 Thr Leu Asp Met Ile Gly Ala Ser xaa xaa Thr Trp Gu xaa Gn His
 260 265 270
 xaa Leu Gly His His Pro Tyr Thr Asn Leu xaa xaa xaa xaa xaa
 275 280 285
 xaa xaa Lys xaa xaa Gly xaa xaa xaa Asp xaa xaa xaa xaa Asp Gn
 290 295 300
 Gu Ser Asp Pro Asp Val Phe Ser xaa Tyr Pro xaa xaa Arg xaa His
 305 310 315 320
 Pro xaa His xaa xaa xaa xaa Tyr His xaa Phe Gn His xaa Tyr xaa
 325 330 335
 Pro xaa xaa Phe xaa xaa Met Thr Ile xaa Lys Val xaa xaa Gn Asp
 340 345 350
 xaa xaa Val xaa xaa xaa xaa Arg Leu xaa xaa Ile Asp Ala xaa xaa
 355 360 365
 Arg Tyr xaa Ser xaa xaa xaa Val xaa Arg Phe Trp xaa Met Lys xaa
 370 375 380
 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 Gu
 405 410 415
 xaa Leu Ala Thr Met Phe Ile Val Asn His xaa Ile Gu Gly Val Ser

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Tyr xaa xaa Lys Asp xaa Val xaa Gly xaa xaa xaa xaa xaa xaa xaa
          435          440          445
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 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 G n Cys G n Thr Ser Val Asn Trp xaa Gly Ser Trp Phe Trp
          500          505          510
Asn His Phe Ser Gly Gly Leu Asn His G n Ile G u His His xaa Phe
          515          520          525
Pro xaa xaa xaa His xaa xaa xaa xaa xaa Ile xaa xaa xaa Val xaa
          530          535          540
xaa Thr Cys xaa G u Tyr Gly Val Pro Tyr G n xaa G u xaa xaa Leu
545          550          555          560
xaa xaa Ala Tyr xaa Lys Met xaa xaa His Leu Arg xaa Leu Gly
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 <223> xaa in position 50 is Phe or Val
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 <223> xaa in position 51 is any amino acid
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 <223> xaa in position 52 is Gln or Ser
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 <223> xaa in position 55 is Ala or Gln

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 20 25 30
 His xaa Phe Pro xaa xaa xaa His xaa xaa xaa xaa Ile xaa xaa
 35 40 45
 xaa xaa xaa xaa Thr Cys xaa Gln Tyr Gly
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 His Gly Ala Phe 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 Gu 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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 xaa xaa Arg Leu xaa xaa Ile Asp
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 35 40 45
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 50 55

<210> 100

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<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>
 <221> Variant
 <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


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<211> 27
<212> PRT
<213> Artificial sequence

<220>
<221> Variant
<222> (2)..(2)
<223> xaa in position 2 is Asp or Gu
<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 Gu 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 Gn 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
Gu xaa Gly xaa xaa xaa Arg xaa xaa xaa Gu
 20           25

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<210> 103
<211> 28
<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> (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 Gu or Gly

<400> 103
G y xaa xaa xaa G u xaa xaa xaa xaa xaa xaa Val Arg xaa Hi s
 1           5           10           15
xaa xaa Pro xaa Asp xaa Trp xaa xaa xaa Hi s xaa
                20                25

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<210> 104
<211> 28
<212> PRT
<213> Artificial sequence

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<220>
<221> Variant

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<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 Gu 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 Gu
 <220>
 <221> Variant
 <222> (28)..(28)
 <223> xaa in position 28 is Asp, Gu or Lys
 <400> 104
 Val Pro Tyr G n xaa G u xaa Ser xaa Leu xaa xaa Ala Tyr xaa Lys
 1 5 10 15
 Met xaa xaa H i s Leu xaa xaa Leu G y xaa xaa xaa
 20 25

<210> 105
 <211> 15
 <212> PRT
 <213> Artificial sequence

<220>
 <221> Variant
 <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
 G y 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 Gu, Asn or Gn
 <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 Gn or Arg
 <220>
 <221> Variant
 <222> (22)..(22)
 <223> xaa in position 22 is Gu or Lys
 <220>
 <221> Variant
 <222> (23)..(23)
 <223> xaa in position 23 is Ala or Gu
 <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 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>
 <221> Variant
 <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

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
 at g agc gcc t cc ggt gcg ct g ct g ccc gcg at c gcg t t c gcc gcg t ac 48
 Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
 1 5 10 15
 gcg t ac gcg acg t ac gcc t ac gcc t t t gag t gg t cg cac gcg aat ggc 96
 Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Gu Trp Ser His Ala Asn Gly
 20 25 30
 at c gac aac gt c gac gcg cgc gag t gg at c ggt gcg ct g t cg t t g agg 144
 Ile Asp Asn Val Asp Ala Arg Gu Trp Ile Gly Ala Leu Ser Leu Arg
 35 40 45
 ct c ccg gcg at c gcg acg acg at g t ac ct g t t g t t c t gc ct g gt c gga 192
 Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
 50 55 60
 ccg agg t t g at g gcg aag cgc gag gcg t t c gac ccg aag ggg t t c at g 240
 Pro Arg Leu Met Ala Lys Arg Gu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75
 ct g gcg t ac aat gcg t at cag acg gcg t t c aac gt c gt c gt g ct c ggg 288
 Leu Ala Tyr Asn Ala Tyr Gn Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95
 at g t t c gcg cga gag at c t cg ggg ct g ggg cag ccc gt g t gg ggg t ca 336
 Met Phe Ala Arg Gu Ile Ser Gly Leu Gly Gn Pro Val Trp Gly Ser
 100 105 110
 acc at g ccg t gg agc gat aga aaa t cg t t t aag at c ct c ct c ggg gt g 384
 Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
 115 120 125
 t gg t t g cac t ac aac aac aaa t at t t g gag ct a t t g gac act gt g t t c 432
 Trp Leu His Tyr Asn Asn Lys Tyr Leu Gu Leu Leu Asp Thr Val Phe
 130 135 140
 at g gt t gcg cgc aag aag acg aag cag t t g agc t t c t t g cac gt t t at 480
 Met Val Ala Arg Lys Lys Thr Lys Gn Leu Ser Phe Leu His Val Tyr
 145 150 155 160
 cat cac gcc ct g t t g at c t gg gcg t gg t gg t t g gt g t gt cac t t g at g 528
 His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
 165 170 175
 gcc acg aac gat t gt at c gat gcc t ac t t c ggc gcg gcg t gc aac t cg 576
 Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
 180 185 190
 t t c att cac at c gt g at g t ac t cg t at t at ct c at g t cg gcg ct c ggc 624
 Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
 195 200 205
 at t cga t gc ccg t gg aag cga t ac at c acc cag gct caa at g ct c caa 672
 Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gn Ala Gn Met Leu Gn
 210 215 220
 t t c gt c att gt c t t c gcg cac gcc gt g t t c gt g ct g cgt cag aag cac 720
 Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gn Lys His
 225 230 235 240
 t gc ccg gt c acc ct t cct t gg gcg caa at g t t c gt c at g acg aac at g 768
 Cys Pro Val Thr Leu Pro Trp Ala Gn Met Phe Val Met Thr Asn Met
 245 250 255
 ct c gt g ct c t t c ggg aac t t c t ac ct c aag gcg t ac t cg aac aag t cg 816
 Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
 260 265 270
 gcg ggc gac ggc gcg agt t cc gt g aaa cca gcc gag acc acg cgc gcg 864
 Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Gu Thr Thr Arg Ala
 275 280 285
 ccc agc gt g cga cgc acg cga t ct cga aaa at t gac t aa 903

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 Gn Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95
 Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gn 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 Gn 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 Gn Ala Gn Met Leu Gn
 210 215 220
 Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gn Lys His
 225 230 235 240
 Cys Pro Val Thr Leu Pro Trp Ala Gn 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 t at ct t ct a gt g ct c t cg gcc gct cgc cag 48
 Met Met Leu Ala Ala Gly Tyr Leu Leu Val Leu Ser Ala Ala Arg Gn
 1 5 10 15
 agc ttc cag cag gac att gac aac ccc aac ggg gcc t ac t cg acc t cg 96
 Ser Phe Gn Gn Asp Ile Asp Asn Pro Asn Gly Ala Tyr Ser Thr Ser
 20 25 30
 t gg act ggc ctg ccc att gt g at g t ct gt g gt c t at ct c agc ggt gt g 144
 Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
 35 40 45
 ttt ggg ct c aca aag t ac ttc gag aac cgg aag ccc at g acg ggg ct g 192
 Sei te 147

PF58307. txt

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		
				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	gtg	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	t aa												834
Ala	Val	Lys	Ala	Glu													
			275														

<210> 112
 <211> 277
 <212> PRT
 <213> Pavlova sp

<400> 112
 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 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
 Sei te 148

PF58307. txt

130 135 140
 H i s Val Tyr H i s H i s Val Leu Leu M e t Tr p A l a Tr p P h e Val Val Val
 145 150 155 160
 L y s Leu G l y A s n G l y A s p A l a Tyr P h e G l y G l y Leu M e t A s n Ser
 165 170 175
 I l e I l e H i s Val M e t M e t Tyr Ser Tyr Tyr Thr M e t A l a Leu Leu G l y
 180 185 190
 Tr p Ser C y s Pro Tr p L y s A r g Tyr Leu Thr G l n A l a G l n Leu Val G l n
 195 200 205
 P h e C y s I l e C y s Leu A l a H i s Ser Thr Tr p A l a A l a Val Thr G l y A l a
 210 215 220
 Tyr Pro Tr p A r g I l e C y s Leu Val G u Val Tr p Val M e t Val Ser M e t
 225 230 235 240
 Leu Val Leu P h e Thr A r g P h e Tyr A r g G l n A l a Tyr A l a L y s G u A l a
 245 250 255
 L y s A l a L y s G u A l a L y s L y s Leu A l a G l n G u A l a Ser G l n A l a L y s
 260 265 270
 A l a Val L y s A l a G u
 275

<210> 113
 <211> 1077
 <212> DNA
 <213> Thal assi osi r a pseudonana

<220>
 <221> CDS
 <222> (1).. (1077)

<400> 113
 a t g t g c t c a t c a c c g c c g t c a c a a t c c a a a a c a a c a t c c c t c c t a g c a 48
 M e t C y s Ser Ser Pro Pro Ser G l n Ser L y s Thr Thr Ser Leu Leu A l a
 1 5 10 15
 c g g t a c a c c a c c g c c c t c c t c c t c a c c c t c a c a a c a t g g t g c 96
 A r g Tyr Thr Thr A l a A l a Leu Leu Leu Leu Thr Leu Thr Thr Tr p C y s
 20 25 30
 c a c t t c g c c t t c c c a g c c g c c a c c g c c a c a c c c g g c c t c a c c g c c g a a 144
 H i s P h e A l a P h e Pro A l a A l a Thr A l a Thr Pro G l y Leu Thr A l a G u
 35 40 45
 a t g c a c t c c t a c a a a g t c c c a c t c g g t c t c a c c g t a t t c t a c c t g c t g 192
 M e t H i s Ser Tyr L y s Val Pro Leu G l y Leu Thr Val P h e Tyr Leu Leu
 50 55 60
 a g t c t a c c g t c a c t a a a g t a c g t t a c g g a c a a c t a c c t t g c c a a a a a g 240
 Ser Leu Pro Ser Leu L y s Tyr Val Thr A s p A s n Tyr Leu A l a L y s L y s
 65 70 75 80
 t a t g a t a t g a a g t c a c t c c t a a c g g a a t c a a t g t g t t g t a c a a t g t g 288
 Tyr A s p M e t L y s Ser Leu Leu Thr G u Ser M e t Val Leu Tyr A s n Val
 85 90 95
 g c g c a a g t g c t g c t c a a t g g g t g g a c g g t g t a t g c g a t t g t g g a t g c g 336
 A l a G l n Val Leu Leu A s n G l y Tr p Thr Val Tyr A l a I l e Val A s p A l a
 100 105 110
 g t g a t g a a t a g a g a c c a t c c g t t t a t t g g a a g t a g a a g t t t g g t t g g g 384
 Val M e t A s n A r g A s p H i s Pro P h e I l e G l y Ser A r g Ser Leu Val G l y
 115 120 125
 g c g g c g t t g c a t a g t g g g a g c t c g t a t g c g g t g t g g t c a t t a t t g t 432
 A l a A l a Leu H i s Ser G l y Ser Ser Tyr A l a Val Tr p Val H i s Tyr C y s
 130 135 140
 g a t a a g t a t t t g g a g t t c t t t g a t a c g t a t t t t a t g g t g t t g a g g g g g 480
 A s p L y s Tyr Leu G u P h e P h e A s p Thr Tyr P h e M e t Val Leu A r g G l y
 145 150 155 160
 a a a a t g g a c c a g g t c t c c t t c c t c c a c a t c t a c c a c c a c a c g a c c a t a 528
 L y s M e t A s p G l n Val Ser P h e Leu H i s I l e Tyr H i s H i s Thr Thr I l e
 165 170 175
 g c g t g g g c a t g g t g g a t c g c c t c c g c t t c t c c c c g g t g g a g a c a t t 576
 A l a Tr p A l a Tr p I l e A l a Leu A r g P h e Ser Pro G l y G l y A s p I l e
 180 185 190
 t a c t t c g g g g c a c t c c t c a a c t c c a t c a t c c a c g t c c t c a t g t a t t c c 624
 Tyr P h e G l y A l a Leu Leu A s n Ser I l e I l e H i s Val Leu M e t Tyr Ser

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G y Cys Thr G y Tyr Thr H i s Tyr Tyr H i s Thr Lys H i s G y A l a Asp
 245 250
 G u Thr G l n P r o Ser Leu G y Thr Tyr Tyr Phe Cys Cys G y Val G l n
 260 265
 Val Phe G u M e t Val Ser Leu Phe Val Leu Phe Ser I l e Phe Tyr Lys
 275 280 285
 Arg Ser Tyr Ser Lys Lys Asn Lys Ser G y G y Lys Asp Ser Lys Lys
 290 295 300
 Asn Asp Asp G y Asn Asn G u Asp G l n Cys H i s Lys A l a M e t Lys Asp
 305 310 315
 I l e Ser G u G y A l a Lys G u Val Val G y H i s A l a A l a Lys Asp A l a
 325 330 335
 G y Lys Leu Val A l a Thr A l a Ser Lys A l a Val Lys Arg Lys G y Thr
 340 345 350
 Arg Val Thr G y A l a M e t
 355

<210> 115
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 <213> *Ostreococcus tauri*

<220>
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 <222> (1)..(903)

<400> 115
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 Met Ser A l a Ser G y A l a Leu Leu P r o A l a I l e A l a Ser A l a A l a Tyr
 1 5 10 15
 gcg t ac gcg acg t ac gcc t ac gcc t t t gag t gg t cg cac gcg aat ggc 96
 A l a Tyr A l a Thr Tyr A l a Tyr A l a Phe 25 G u Trp Ser H i s A l a Asn G y
 20 30
 at c gac aac gt c gac gcg cgc gag t gg at c ggt gcg ct g t cg t t g agg 144
 I l e Asp Asn Val Asp A l a Arg G u Trp I l e G y A l a Leu Ser Leu Arg
 35 40 45
 ct c ccg gcg at c gcg acg acg at g t ac ct g t t g t t c t gc ct g gt c gga 192
 Leu P r o A l a I l e A l a Thr Thr Met Tyr Leu Leu Phe Cys Leu Val G y
 50 55 60
 ccg agg t t g at g gcg aag cgc gag gcg t t c gac ccg aag ggg t t c at g 240
 P r o Arg Leu M e t A l a Lys Arg G u A l a Phe Asp P r o Lys G y Phe M e t
 65 70 75 80
 ct g gcg t ac aat gcg t at cag acg gcg t t c aac gt c gt c gt g ct c ggg 288
 Leu A l a Tyr Asn A l a Tyr G l n Thr A l a Phe Asn Val Val Val Leu G y
 85 90 95
 at g t t c gcg cga gag at c t cg ggg ct g ggg cag ccc gt g t gg ggg t ca 336
 Met Phe A l a Arg G u I l e Ser G y Leu G y G l n P r o Val Trp G y Ser
 100 105 110
 acc at g ccg t gg agc gat aga aaa t cg t t t aag at c ct c ct c ggg gt g 384
 Thr M e t P r o Trp Ser Asp Arg Lys Ser Phe Lys I l e Leu Leu G y Val
 115 120 125
 t gg t t g cac t ac aac aac aaa t at t t g gag ct a t t g gac act gt g t t c 432
 Trp Leu H i s Tyr Asn Asn Lys Tyr Leu G u Leu Leu Asp Thr Val Phe
 130 135 140
 at g gt t gcg cgc aag aag acg aag cag t t g agc t t c t t g cac gt t t at 480
 Met Val A l a Arg Lys Lys Thr Lys G l n Leu Ser Phe Leu H i s Val Tyr
 145 150 155 160
 cat cac gcc ct g t t g at c t gg gcg t gg t gg t t g gt g t gt cac t t g at g 528
 H i s H i s A l a Leu Leu I l e Trp A l a Trp Trp Leu Val Cys H i s Leu M e t
 165 170 175
 gcc acg aac gat t gt at c gat gcc t ac t t c ggc gcg gcg t gc aac t cg 576
 A l a Thr Asn Asp Cys I l e Asp A l a Tyr Phe G y A l a A l a Cys Asn Ser
 180 185 190
 t t c at t cac at c gt g at g t ac t cg t at t at ct c at g t cg gcg ct c ggc 624
 Phe I l e H i s I l e Val M e t Tyr Ser Tyr Tyr Leu M e t Ser A l a Leu G y
 195 200 205
 at t cga t gc ccg t gg aag cga t ac at c acc cag gct caa at g ct c caa 672
 I l e Arg Cys P r o Trp Lys Arg Tyr I l e Thr G l n A l a G l n M e t Leu G l n

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<223> xaa in position 53 to 73 is any amino acid
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<223> xaa in position 74 to 79 is any or no amino acid
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<223> xaa in position 85 to 86 is any amino acid
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<222> (88)..(90)
<223> xaa in position 88 to 90 is any amino acid
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<222> (112)..(127)
<223> xaa in position 112 to 127 is any amino acid
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<222> (128)..(128)
<223> xaa in position 128 is any or no amino acid
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<222> (130)..(130)
<223> xaa in position 130 is any amino acid
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<222> (133)..(134)
<223> xaa in position 133 to 134 is any amino acid
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<222> (137)..(137)
<223> xaa in position 137 is any amino acid
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<222> (139)..(140)
<223> xaa in position 139 to 140 is any amino acid
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<223> xaa in position 143 to 144 is any amino acid
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<222> (147)..(147)
<223> xaa in position 147 is any amino acid

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<222> (151)..(152)
<223> xaa in position 151 to 152 is any amino acid
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<222> (154)..(154)
<223> xaa in position 154 is any amino acid
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<222> (159)..(159)
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<222> (163)..(166)
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<222> (180)..(182)
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<222> (184)..(184)
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<222> (193)..(193)
<223> xaa in position 193 is any amino acid
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<222> (196)..(197)
<223> xaa in position 196 to 197 is any amino acid
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<222> (203)..(206)
<223> xaa in position 203 to 206 is any amino acid
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<222> (208)..(210)
<223> xaa in position 208 to 210 is any amino acid
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<223> xaa in position 217 is any amino acid
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<222> (222)..(223)
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<223> xaa in position 226 to 252 is any amino acid
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<222> (253)..(267)
<223> xaa in position 253 to 267 is any or no amino acid

<220>
 <221> Vari ant
 <222> (269).. (272)
 <223> xaa in position 269 to 272 is any ami no aci d
 <220>
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 <222> (276).. (277)
 <223> xaa in position 276 to 277 is any ami no aci d
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 <221> Vari ant
 <222> (280).. (282)
 <223> xaa in position 280 to 282 is any ami no aci d
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 <222> (284).. (291)
 <223> xaa in position 284 to 291 is any ami no aci d
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 <221> Vari ant
 <222> (292).. (324)
 <223> xaa in position 292 to 324 is any or no ami no aci d
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 <221> Vari ant
 <222> (326).. (337)
 <223> xaa in position 326 to 337 is any ami no aci d
 <220>
 <221> Vari ant
 <222> (338).. (338)
 <223> xaa in position 338 is any or no ami no aci d

<400> 117
 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
 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 G n xaa xaa xaa xaa xaa xaa xaa xaa xaa
 85 90 95
 xaa 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 Hi s Tyr xaa xaa Lys Tyr xaa G u xaa xaa Asp Thr xaa xaa
 130 135 140
 Met Val xaa Arg xaa Lys xaa xaa G n xaa Ser Phe Leu Hi s xaa Tyr
 145 150 155 160
 Hi s Hi s xaa xaa xaa xaa Trp Al a Trp xaa xaa xaa xaa xaa xaa
 165 170 175
 xaa xaa xaa xaa xaa xaa Asp xaa Tyr Phe G y xaa xaa xaa Asn Ser
 180 185 190
 xaa Ile Hi s xaa xaa Met Tyr Ser Tyr Tyr xaa xaa xaa xaa Leu xaa
 195 200 205
 xaa xaa Oys Pro Trp Lys Arg Tyr xaa Thr G n Al a G n xaa xaa G n
 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

<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 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>
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 <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
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 <221> Variant
 <222> (12)..(12)
 <223> xaa in position 12 is any amino acid
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 <221> Variant
 <222> (13)..(14)
 <223> xaa in position 13 to 14 is any or no amino acid
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 <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
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 <221> Variant
 <222> (25)..(26)
 <223> xaa in position 25 to 26 is any amino acid
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 <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
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 <221> Variant
 <222> (39)..(39)
 <223> xaa in position 39 is Ile or Leu
 <220>
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 <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>
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 <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
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 <221> Variant
 <222> (10)..(10)
 <223> xaa in position 10 is any amino acid
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 <221> Variant
 <222> (13)..(14)
 <223> xaa in position 13 to 14 is any amino acid
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<222> (15)..(15)
<223> xaa in position 15 is Ala or Ser
<220>
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<222> (17)..(17)
<223> xaa in position 17 is Ala or Ser
<220>
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<222> (18)..(18)
<223> xaa in position 18 is any amino acid
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<222> (19)..(19)
<223> xaa in position 19 is Gu or Lys
<220>
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<222> (20)..(20)
<223> xaa in position 20 is Ala, Asn or Ser
<220>
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<222> (21)..(21)
<223> xaa in position 21 is Lys or Arg
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<222> (22)..(22)
<223> xaa in position 22 is Ala, Gly or Ser
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<223> xaa in position 23 is any amino acid
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<222> (24)..(24)
<223> xaa in position 24 is Gu or Gly
<220>
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<222> (25)..(29)
<223> xaa in position 25 to 29 is any amino acid
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<222> (30)..(30)
<223> xaa in position 30 is Asn, Pro or Gn
<220>
<221> Variant
<222> (31)..(32)
<223> xaa in position 31 to 32 is Ala, Asp or Gu
<220>
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<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, Gn or Thr
<220>
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<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

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20
xaa xaa xaa xaa xaa
35

<210> 121
<211> 19
<212> PRT
<213> Artificial sequence

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<222> (4)..(7)
<223> xaa in position 4 to 7 is any or no amino acid
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<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
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<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
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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

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20 25 30
 cag tgg gat atc gga cct gtt tct tct tct acc gct cat ttg cct gct 144
 G n Trp Asp lle Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
 35
 att gag tct cct act cct ttg gtg acc tct ttg ctg ttc tac ttg gtg 192
 lle Gu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
 50
 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
 atc aga gag cct acc tgg ttg agg aga ttc atc atc tgc cac aac gct 288
 lle Arg Glu Pro Thr Trp Leu Arg Arg Phe lle lle Cys His Asn Ala
 85
 ttc ttg att gtg ctg tcc ttg tac atg tgt ttg gga tgc gtt gct caa 336
 Phe Leu lle Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala G n
 100
 gct tac caa aac gga tac acc ttg tgg gga aac gag ttc aag gct act 384
 Ala Tyr G n Asn Gly Tyr Thr Leu Trp Gly Asn Gu Phe Lys Ala Thr
 115
 gag acc caa ttg gct ctg tac atc tac atc ttc tac gtg tcc aag atc 432
 Gu Thr G n Leu Ala Leu Tyr lle Tyr lle Phe Tyr Val Ser Lys lle
 130
 tac gag ttc gtg gat acc tac atc atg ctg ctg aag aac ctg agg 480
 Tyr Gu Phe Val Asp Thr Tyr lle Met Leu Leu Lys Asn Asn Leu Arg
 145
 caa gtg tct ttc ttg cac atc tac cac cac tct acc atc tct ttc atc 528
 G n Val Ser Phe Leu His lle Tyr His His Ser Thr lle Ser Phe lle
 165
 tgg tgg atc atc gct aga aga gca cct gga gga gat gct tat ttc tcc 576
 Trp Trp lle lle Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
 180
 gct gct ctg aac tct tgg gtt cat gtg tgc atg tac act tac tcc ctg 624
 Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
 195
 ctg tct acc ttg att gga aag gaa gat cct aag agg tct aac tac ctg 672
 Leu Ser Thr Leu lle Gly Lys Gu Asp Pro Lys Arg Ser Asn Tyr Leu
 210
 tgg tgg gga agg cat ttg acc caa atg caa atg ctg cag ttc ttc ttc 720
 Trp Trp Gly Arg His Leu Thr G n Met G n Met Leu G n Phe Phe Phe
 225
 aac gtg ctg caa gct ctt tat tgc gct tcc ttc tcc act tac cct aag 768
 Asn Val Leu G n Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro aag
 245
 ttc ctg tcc aag atc ttg ctg gtg tac atg atg tct ttg ctg gga ctt 816
 Phe Leu Ser Lys lle Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
 260
 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 lle Ala Ala Ala Lys Leu G n
 275
 aag aag cag cag tga 879
 Lys Lys G n G n
 290

<210> 123
 <211> 292
 <212> PRT
 <213> *Ostreococcus tauri*

<400> 123
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 1 5 10
 Trp Asp Tyr Ala lle Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
 20 25 30
 G n Trp Asp lle Gly Pro Val Ser Ser Thr Ala His Leu Pro Ala
 35 40 45
 lle Gu 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
 Sei te 161

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65 Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
 85 Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gn
 100 Ala Tyr Gn Asn Gly Tyr Thr Leu Trp Gly Asn Gu Phe Lys Ala Thr
 115 Gu Thr Gn Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
 130 Tyr Gu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
 145 Gn Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
 165 Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
 180 Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
 195 Leu Ser Thr Leu Ile Gly Lys Gu Asp Pro Lys Arg Ser Asn Tyr Leu
 210 Trp Trp Gly Arg His Leu Thr Gn Met Gn Met Leu Gn Phe Phe Phe
 225 Asn Val Leu Gn Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
 245 Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu
 260 Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gn
 275 Lys Lys Gn Gn
 290

<210> 124
 <211> 1047
 <212> DNA
 <213> Marchantia polymorpha

<220>
 <221> CDS
 <222> (1)..(1047)

<400> 124
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 Met Ala Thr Lys Ser Gly Ser Gly Leu Leu Glu Trp Ile Ala Val Ala
 15
 gcg aag atg aag caa gct cgg agc agc ccc gag ggt gag atc gtg ggt 96
 Ala Lys Met Lys Gn Ala Arg Ser Ser Pro Glu Gly Gu 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 Gu 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 ctg gag gag aga atc ggg gag gcg gtg ttc aga gtt 240
 Lys Val Arg Pro Leu Gu Gu Arg Ile Gly Gu 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 Gu 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 Gu Ser Pro 105 Val Pro Val Leu Ala Cys Ile
 100 110
 tct ctg tac ttg ctg 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 ctg gcc ctg cgt tgc ctt 432
 Ser Gly Gn Lys Pro Arg Lys Gu 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

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Val 145	I l e	A l a	H i s	Asn 150	Leu 150	P h e	Leu	C y s	C y s	Leu 155	S e r	Leu	P h e	M e t	C y s 160	
g t c	g g t	c t c	a t t	g c c	g c a	g c t	c g a	c a t	t a c	g g g	t a t	a g t	g t a	t g g	g g g	528
Val	G y	Leu	I l e	A l a 165	A l a	A l a	A r g	H i s	T y r	G y	T y r	S e r	V a l	T r p	G y	
a a c	t a c	t a c	a g a	g a a	a g a	g a a	c c c	g c a	a t g	a a t	t t g	c t c	a t t	t a c	g t g	576
Asn	T y r	T y r	A r g	G u	A r g	G u	P r o	A l a 185	M e t	Asn	Leu	Leu	I l e	T y r	V a l	
t t c	t a c	a t g	t c g	a a g	c t g	t a c	g a a	t t t	a t g	g a c	a c g	g c c	a t t	a t g	t t a	624
P h e	T y r	M e t	S e r	L y s	Leu	T y r	G u 200	P h e	M e t	Asp	Thr	A l a	I l e	M e t	Leu	
t t c	a g a	a g a	a a t	c t g	c g a	c a a	g t c	a c g	t a c	t t g	c a t	g t a	t a t	c a c	c a c	672
P h e	A r g	A r g	Asn	Leu	A r g	G n 215	V a l	Thr	T y r	Leu	H i s	V a l	T y r	H i s	H i s	
g c a	a g c	a t c	g c a	a t g	a t t	t g g	t g g	a t a	a t t	t g c	t a t	c g g	t t t	c c a	g g a	720
A l a	S e r	I l e	A l a	M e t	I l e	T r p	T r p	I l e	I l e	C y s	T y r	A r g	P h e	P r o	G y	
225				230						235					240	
g c t	g a t	t c g	t a t	t t c	t c c	g c a	g c a	t t c	a a t	t c c	t g t	a t c	c a t	g t a	g c g	768
A l a	A s p	S e r	T y r	P h e 245	S e r	A l a	A l a	P h e	Asn 250	S e r	C y s	I l e	H i s	V a l	A l a	
a t g	t a c	c t g	t a t	t a t	c t a	c t c	g c g	g c a	a c c	g t c	g c c	a g a	g a c	g a a	a a g	816
M e t	T y r	Leu	T y r 260	T y r	Leu	Leu	A l a	A l a	Thr 265	V a l	A l a	A r g	Asp 270	G u	L y s	
c g g	a g a	c g c	aaa	t a t	c t c	t t c	t g g	g g a	a a g	t a t	c t g	a c c	a t c	a t a	c a a	864
A r g	A r g	A r g	L y s	T y r	Leu	P h e	T r p 280	G y	L y s	T y r	Leu	Thr 285	I l e	I l e	G n	
a t g	c t t	c a g	t t t	t t g	t c c	t t c	a t t	g g g	c a g	g c g	a t t	t a t	g c a	a t g	t g g	912
M e t	Leu 290	G n	P h e	Leu	S e r	P h e 295	I l e	G y	G n	A l a	I l e	T y r	A l a	M e t	T r p	
a a g	t t t	g a a	t a c	t a t	c c c	a a g	g g c	t t t	g g c	a g g	a t g	t t g	t t c	t t t	t a c	960
L y s	P h e	G u	T y r	T y r	P r o 310	L y s	G y	P h e	G y	A r g 315	M e t	Leu	P h e	P h e	T y r 320	
t c t	g t a	t c a	t t g	t t g	g c a	t t t	t t c	g g c	a a c	t t c	t t t	g t c	aaa	a a g	t a t	1008
S e r	V a l	S e r	Leu	Leu	A l a 325	P h e	P h e	G y	Asn 330	P h e	P h e	V a l	L y s	L y s	T y r 335	
t c g	a a c	g c t	t c a	c a g	c c t	a a g	a c a	g t t	aaa	g t g	g a g	t g a				1047
S e r	Asn	A l a	S e r 340	G n	P r o	L y s	Thr	V a l 345	L y s	V a l	G u					

<210> 125
 <211> 348
 <212> PRT
 <213> M a r c h a n t i a p o l y m o r p h a

<400> 125

M e t	A l a	Thr	L y s	S e r	G y	S e r	G y	Leu	Leu	G u	T r p	I l e	A l a	V a l	A l a
1				5				10						15	
A l a	L y s	M e t	L y s	G n	A l a	A r g	S e r	S e r	P r o	G u	G y	G u	I l e	V a l	G y
			20					25					30		
G y	Asn	A r g	M e t	G y	S e r	G y	Asn	G y	A l a	G u	T r p	Thr	Thr	S e r	Leu
			35				40					45			
I l e	H i s	A l a	P h e	Leu	Asn	A l a	Thr	Asn	G y	L y s	S e r	G y	G y	A l a	S e r
					55						60				
L y s	V a l	A r g	P r o	Leu	G u	G u	A r g	I l e	G y	G u	A l a	V a l	P h e	A r g	V a l
65				70						75					80
Leu	G u	A s p	V a l	V a l	G y	V a l	A s p	I l e	A r g	L y s	P r o	Asn	P r o	V a l	Thr
				85					90					95	
L y s	A s p	Leu	P r o	M e t	V a l	G u	S e r	P r o	V a l	P r o	V a l	Leu	A l a	C y s	I l e
			100					105						110	
S e r	Leu	T y r	Leu	Leu	V a l	V a l	T r p	Leu	T r p	S e r	S e r	H i s	I l e	L y s	A l a
							120					125			
S e r	G y	G n	L y s	P r o	A r g	L y s	G u	A s p	P r o	Leu	A l a	Leu	A r g	C y s	Leu
						135					140				
V a l	I l e	A l a	H i s	Asn	Leu	P h e	Leu	C y s	C y s	Leu	S e r	Leu	P h e	M e t	C y s
145				150						155					160
V a l	G y	Leu	I l e	A l a	A l a	A l a	A r g	H i s	T y r	G y	T y r	S e r	V a l	T r p	G y
				165					170					175	
Asn	T y r	T y r	A r g	G u	A r g	G u	P r o	A l a	M e t	Asn	Leu	Leu	I l e	T y r	V a l

PF58307. txt

ggc t ac t ac gcc t cg acc acc ct c ggc t ac acc t t c at g cg c ccg ct g 624
 G y Tyr Tyr Al a Ser Thr Thr Leu G y Tyr Thr Phe Met Arg Pro Leu
 195 200 205
 cg c ccg t ac att acc acc att ct c acg cag t t c at g gcc at g gt c 672
 Arg Pro Tyr Ile Thr Thr Ile G n Leu Thr G n Phe Met Al a Met Val
 210 215 220
 gt c cag t cc gt c t at gac t ac t ac aac ccc t gc gac t ac ccg cag ccc 720
 Val G n Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro G n Pro
 225 230 240
 ct c gt c aag ct g ct c t t c t gg t ac at g ct c acc at g ct c gcc ct c t t c 768
 Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu G y Leu Phe
 245 250 255
 ggc aac t t c t t c gt g cag cag t ac ct c aag ccc aag gcg ccc aag aag 816
 G y Asn Phe Phe Val G n G n Tyr Leu Lys Pro Lys Al a Pro Lys Lys
 260 270
 cag aag acc at c t aa 831
 G n Lys Thr Ile
 275

<210> 127
 <211> 276
 <212> PRT
 <213> Thraustochytrium sp

<400> 127
 Met Asp Val Val G u G n G n Trp Arg Arg Phe Val Asp Al a Val Asp
 1 5 10
 Asn G y Ile Val G u Phe Met G u His G u G u Pro Asn Lys Leu Asn
 20 25 30
 G u G y Lys Leu Ser Thr Ser Thr G u G u Met Met Al a Leu Ile Val
 35 40 45
 G y Tyr Leu Al a Phe Val Val Leu G y Ser Al a Phe Met Lys Al a Phe
 50 55 60
 Val Asp Lys Pro Phe G u Leu Lys Phe Leu Lys Leu Val His Asn Ile
 65 70 75 80
 Phe Leu Thr G y Leu Ser Met Tyr Met Al a Thr G u Cys Al a Arg G n
 85 90 95
 Al a Tyr Leu G y G y Tyr Lys Leu Phe G y Asn Pro Met G u Lys G y
 100 105 110
 Thr G u Ser His Al a Pro G y Met Al a Asn Ile Ile Tyr Ile Phe Tyr
 115 120
 Val Ser Lys Phe Leu G u Phe Leu Asp Thr Val Phe Met Ile Leu G y
 130 135 140
 Lys Lys Trp Lys G n Leu Ser Phe Leu His Val Tyr His His Al a Ser
 145 150 155 160
 Ile Ser Phe Ile Trp G y Ile Ile Al a Arg Phe Al a Pro G y G y Asp
 165 170 175
 Al a Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
 180 185 190
 G y Tyr Tyr Al a Ser Thr Thr Leu G y Tyr Thr Phe Met Arg Pro Leu
 195 200 205
 Arg Pro Tyr Ile Thr Thr Ile G n Leu Thr G n Phe Met Al a Met Val
 210 215 220
 Val G n Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro G n Pro
 225 230 235 240
 Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu G y Leu Phe
 245 250 255
 G y Asn Phe Phe Val G n G n Tyr Leu Lys Pro Lys Al a Pro Lys Lys
 260 270
 G n Lys Thr Ile
 275

<210> 128
 <211> 1146
 <212> DNA
 <213> Leishmani a major

<220>

<221> CDS

<222> (1)..(1146)

<400> 128

at g	gt c	t ct	ct g	gag	cag	gcc	gaa	cag	at c	gcg	gcc	gcg	at c	gag	gt a	48
Met	Val	Ser	Leu	Gl u	Gl n	Al a	Gl u	Gl n	I le	Al a	Al a	Al a	I le	Gl u	Val	
1			5					10					15			
cct	gac	t gg	gt c	tt g	aca	aag	t ct	gcg	gcg	ct g	gt g	t ac	agc	t gc	tt c	96
Pro	Asp	Trp	Val	Leu	Thr	Lys	Ser	Al a	Al a	Leu	Val	Tyr	Ser	Cys	Phe	
			20					25					30			
ggc	t cc	gcc	gcc	aat	gct	tt t	gaa	agc	agt	at c	aag	at c	aac	tt c	ccg	144
Gl y	Ser	Al a	Al a	Asn	Al a	Phe	Gl u	Ser	Ser	I le	Lys	I le	Asn	Phe	Pro	
			35				40					45				
gcg	cag	cat	gcg	tt c	gt g	gag	gcg	t gg	at g	cg c	gcg	cg c	t cc	cac	cct	192
Al a	Gl n	Hi s	Al a	Phe	Val	Gl u	Al a	Trp	Met	Arg	Al a	Arg	Ser	Hi s	Pro	
			50				55					60				
tt t	gcg	gag	cg c	ct g	ccg	t ac	ct g	aat	ccg	t gg	cac	gt t	at c	gcc	t cg	240
Phe	Al a	Gl u	Arg	Leu	Pro	Tyr	Leu	Asn	Pro	Trp	Hi s	Val	I le	Al a	Ser	
			65				70			75				80		
at a	ct g	gcc	t ac	ct c	t cc	tt g	att	gt c	acc	tt g	cg c	ct g	tt g	cat	cgt	288
I le	Leu	Al a	Tyr	Leu	Ser	Leu	I le	Val	Thr	Leu	Arg	Leu	Leu	Hi s	Arg	
			85					90						95		
gt a	ct c	ggt	aag	tt c	t cg	t gc	cg c	act	ct c	gga	tt g	gt g	cac	aac	ct c	336
Val	Leu	Gl y	Lys	Phe	Ser	Cys	Arg	Thr	Leu	Gl y	Leu	Val	Hi s	Asn	Leu	
			100					105					110			
ggt	ct c	cat	ct t	ct c	t cg	tt g	t ac	at g	agc	ct t	ggt	ct c	at g	at c	agc	384
Gl y	Leu	Hi s	Leu	Leu	Ser	Leu	Tyr	Met	Ser	Leu	Gl y	Leu	Met	I le	Ser	
			115				120					125				
gcg	cg c	gcc	gcg	ggg	t ac	t cg	ct c	t gg	aac	aac	gcg	gt c	ggc	acc	t cc	432
Al a	Arg	Al a	Al a	Gl y	Tyr	Ser	Leu	Trp	Asn	Asn	Al a	Val	Gl y	Thr	Ser	
			130				135				140					
ccg	gct	gag	t gg	cg c	att	gcg	aag	ct g	at c	t gg	ct c	tt c	t at	gt c	t cg	480
Pro	Al a	Gl u	Trp	Arg	I le	Al a	Lys	Leu	I le	Trp	Leu	Phe	Tyr	Val	Ser	
			145				150			155					160	
aag	gt g	gt g	gaa	t gg	gt g	gac	acg	gt a	att	at g	tt a	tt a	aag	cag	aac	528
Lys	Val	Val	Gl u	Trp	Val	Asp	Thr	Val	I le	Met	Leu	Leu	Lys	Gl n	Asn	
			165					170						175		
t ac	cac	cag	gt c	acc	tt c	ct g	cac	gt g	t at	cac	cac	acg	acg	gt t	tt t	576
Tyr	Hi s	Gl n	Val	Thr	Phe	Leu	Hi s	Val	Tyr	Hi s	Hi s	Thr	Thr	Val	Phe	
			180					185						190		
gt g	ct g	t gg	t gg	ct g	gcg	tt g	ct g	gt c	cct	ggc	ggc	ggc	gag	t cg	t ac	624
Val	Leu	Trp	Trp	Leu	Al a	Leu	Leu	Val	Al a	Pro	Gl y	Gl y	Gl u	Ser	Tyr	
			195				200					205				
t ac	agc	gcc	at g	gt g	aac	t ct	ggc	gt c	cac	gt t	tt c	at g	t ac	ggg	t ac	672
Tyr	Ser	Al a	Met	Val	Asn	Ser	Gl y	Val	Hi s	Val	Phe	Met	Tyr	Gl y	Tyr	
			210				215					220				
t ac	tt t	ct c	acg	ct g	ct c	tt c	cca	t cc	ggc	at c	gt g	cg c	gac	gt c	tt g	720
Tyr	Phe	Leu	Thr	Leu	Leu	Phe	Pro	Ser	Gl y	I le	Val	Arg	Asp	Val	Leu	
			225				230					235			240	
agc	aag	tt c	aag	tt t	gcc	att	acg	aag	ggc	cag	at g	t gg	cag	tt c	gt c	768
Ser	Lys	Phe	Lys	Phe	Al a	I le	Thr	Lys	Gl y	Gl n	Met	Trp	Gl n	Phe	Val	
			245						250					255		
tt c	aac	t gc	ct a	cag	t cc	gcg	t ac	gac	ct c	gt g	t gg	gt g	ccg	cg g	gaa	816
Phe	Asn	Cys	Leu	Gl n	Ser	Al a	Tyr	Asp	Leu	Val	Trp	Val	Pro	Arg	Gl u	
			260					265								
gag	ct c	aag	t ac	agc	gcg	gag	ct g	ct g	cag	at c	ct c	tt c	t gg	t ac	at g	864
Gl u	Leu	Lys	Tyr	Ser	Al a	Gl u	Leu	Leu	Gl n	I le	Leu	Phe	Trp	Tyr	Met	
			275				280						285			
at c	t cc	ct c	tt g	gcg	ct c	tt t	ggc	aac	tt c	tt g	gt g	aag	aac	aag	aag	912
I le	Ser	Leu	Leu	Al a	Leu	Phe	Gl y	Asn	Phe	Leu	Val	Lys	Asn	Lys	Lys	
			290				295					300				
tt c	t cg	cac	cg c	cg c	t gc	gt t	gat	gcc	gcg	act	gct	t cg	ggc	gcg	aag	960
Phe	Ser	Hi s	Arg	Arg	Cys	Val	Asp	Al a	Al a	Thr	Al a	Ser	Gl y	Al a	Lys	
			305				310				315				320	
gag	gac	acg	gcg	gcg	agg	t cc	cac	ggc	gac	cg c	acc	cac	aga	acc	cgt	1008
Gl u	Asp	Thr	Al a	Al a	Arg	Ser	Hi s	Gl y	Asp	Arg	Thr	Hi s	Arg	Thr	Arg	
			325						330					335		
gt g	aag	gct	ggc	at g	acc	aac	at g	caa	ct g	gag	agg	ct g	aag	aat	gag	1056

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Val	Lys	Ala	Gly	Met	Thr	Asn	Met	Gln	Leu	Glu	Arg	Leu	Lys	Asn	Glu		
aag	tcc	acg	gag	atg	aag	ctg	ctg	atg	cgc	aag	aac	ggc	aac	ggc	aac		
Lys	Ser	Thr	Glu	Met	Lys	Leu	Leu	Met	Arg	Lys	Asn	Gly	Asn	Gly	Asn		1104
		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> Leishmaniama 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	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

<220>
 <221> CDS
 <222> (1)..(879)

<400> 130
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 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 Thr Ala His Leu Pro Ala
 35 40 45
 att gaa tcc cct acc cca ctg gtg act agc ctg ttt 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
 att aga gag cct acg ttg 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
 80 85 90
 ttc ttg ata gtc ctg 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
 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 ctg 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 ctg 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 ctg 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 cgg 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 ctg 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 ctg 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 ctg gtc tat atg atg agc ctt ctg 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 ctg 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*

PF58307. txt

<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 Ile Val Leu
 35 40 45
 ggg ttg tcg tcc tac ttg aca ttc gtg ttt ctg ggg ctg 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 ctg agt ctg tac 288
 Leu Phe Val Ile Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr

Seite 169

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      85          90          95
at g tgc gt g gga at t gt c cgt caa gct at c ct c aac agg t ac t ct ct g      336
Met Cys Val Gly Ile Val Arg Gn Ala Ile Leu Asn Arg Tyr Ser Leu
      100
t gg ggc aat gcg t ac aat ccc aaa gaa gt t caa at g ggc cac ct g ct c      384
Trp Gy Asn Ala Tyr Asn Pro Lys Gu Val Gn Met Gy His Leu Leu
      115
t ac att ttc t ac at g t ca aag t ac at c gag ttt at g gac acg gt c at t      432
Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Gu Phe Met Asp Thr Val Ile
      130
at g att ttg aag cgc aac acg cgc cag at c act gt g tt g cat gt g t ac      480
Met Ile Leu Lys Arg Asn Thr Arg Gn Ile Thr Val Leu His Val Tyr
      145
cac cac gca tcc at c tcc ttc at c tgg tgg at c at c gcc t ac cat gct      528
His His Ala Ser Ile Ser Phe Ile Trp Trp Ile Ile Ala Tyr His Ala
      165
cct ggc ggt gaa gct t at ttc tct gcc gca ttg aac tcc gga gt a cat      576
Pro Gy Gy Gu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gy Val His
      180
gt g ct c at g t ac ct c t ac t ac ctt ttg gca gca act ct g gga aag aac      624
Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gy Lys Asn
      195
gag aaa gct cgc cgc aag t ac ct a tgg tgg gga aaa t ac ttg aca cag      672
Gu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gy Lys Tyr Leu Thr Gn
      210
ct g cag at g ttc cag ttt gt c ctt aac at g att cag gct t ac t ac gat      720
Leu Gn Met Phe Gn Phe Val Leu Asn Met Ile Gn Ala Tyr Tyr Asp
      225
att aag aac aac t cg cct t ac cca caa ttt ttg at c cag att ttg ttc      768
Ile Lys Asn Asn Ser Pro Tyr Pro Gn Phe Leu Ile Gn Ile Leu Phe
      245
t ac t ac at g at c t cg ctt tta gcg ct a ttt gga aac ttt t ac gt t cac      816
Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gy Asn Phe Tyr Val His
      260
aaa t ac gt a t ca gcg ccc gca aaa cct gcg aag at c aag agc aaa aag      864
Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
      275
gca gaa t aa
Ala Gu
      290

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<210> 133
 <211> 290
 <212> PRT
 <213> Marchantia polymorpha

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<400> 133
Met Gu Ala Tyr Gu Met Val Asp Ser Phe Val Ser Lys Thr Val Phe
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Gu Thr Leu Gn Arg Leu Arg Gy Gy Val Val Leu Thr Gu Ser Ala
20 25 30
Ile Thr Lys Gy Leu Pro Cys Val Asp Ser Pro Thr Pro Ile Val Leu
35 40 45
Gy Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gy Leu Ile Val Ile
50 55 60
Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Gu 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 Gy Ile Val Arg Gn Ala Ile Leu Asn Arg Tyr Ser Leu
100 105 110
Trp Gy Asn Ala Tyr Asn Pro Lys Gu Val Gn Met Gy His Leu Leu
115 120 125
Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Gu Phe Met Asp Thr Val Ile
130 135 140
Met Ile Leu Lys Arg Asn Thr Arg Gn 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

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PF58307. txt

165
 Pro Gly Gly Gu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
 170
 Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gly Lys Asn
 180
 195
 200
 205
 210
 215
 220
 225
 230
 235
 240
 245
 250
 255
 260
 265
 270
 275
 280
 285
 Ala Gu
 290

<210> 134
 <211> 873
 <212> DNA
 <213> Physcomitrella patens

<220>
 <221> CDS
 <222> (1).. (873)

<400> 134
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 1
 5
 10
 15
 cag ggc gt g aat gca t t g ct g ggt agt t t t ggg gt g gag t t g acg gat 96
 Gn Gy Val Asn Ala Leu Leu Gy Ser Phe Gy Val Gu Leu Thr Asp
 20
 25
 30
 acg ccc act acc aaa ggc t t g ccc ct c gt t gac agt ccc aca ccc at c 144
 Thr Pro Thr Lys Gy Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
 35
 40
 45
 gt c ct c ggt gt t t ct gt a t ac t t g act at t gt c at t gga ggg ct t t t g 192
 Val Leu Gy Val Ser Val Tyr Leu Thr Ile Val Ile Gy Gy Leu Leu
 50
 55
 60
 t gg at a aag gcc agg gat ct g aaa ccg cgc gcc t cg gag cca t t t t t g 240
 Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Gu Pro Phe Leu
 65
 70
 75
 80
 ct c caa gct t t g gt g ct t gt g cac aac ct g t t c t gt t t t gcg ct c agt 288
 Leu Gn Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
 85
 90
 95
 ct g t at at g t gc gt g ggc at c gct t at cag gct at t acc t gg cgg t ac 336
 Leu Tyr Met Cys Val Gy Ile Ala Tyr Gn Ala Ile Thr Trp Arg Tyr
 100
 105
 110
 t ct ct c t gg ggc aat gca t ac aat cct aaa cat aaa gag at g gcg at t 384
 Ser Leu Trp Gy Asn Ala Tyr Asn Pro Lys His Lys Gu Met Ala Ile
 115
 120
 125
 ct g gt a t ac t t g t t c t ac at g t ct aag t ac gt g gaa t t c at g gat acc 432
 Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Gu Phe Met Asp Thr
 130
 135
 140
 gt t at c at g at a ct g aag cgc agc acc agg caa at a agc t t c ct c cac 480
 Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gn Ile Ser Phe Leu His
 145
 150
 155
 160
 gt t t at cat cat t ct t ca at t t cc ct c at t t gg t gg gct at t gct cat 528
 Val Tyr His His Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
 165
 170
 175
 cac gct cct ggc ggt gaa gca t at t gg t ct gcg gct ct g aac t ca gga 576
 His Ala Pro Gy Gy Gu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gy
 180
 185
 190
 195
 gt g cat gt t ct c at g t at gcg t at t ac t t c t t g gct gcc t gc ct t cga 624
 Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
 200
 205
 agt agc cca aag t t a aaa aat aag t ac ct t t t t gg ggc agg t ac t t g 672

PF58307.txt

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 Gn Phe Gn Met Phe Gn Phe Met Leu Asn Leu Val Gn Ala Tyr
 225 230 240
 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 Gn Trp Leu Ile Lys Ile
 245 250 255
 ttg ttc tac tac atg atc tcg ttg ctg ttt ctg 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 Gn Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gn Lys Gly Ala Lys
 275 280 285
 act gag tga 873
 Thr Gu
 290

<210> 135
 <211> 290
 <212> PRT
 <213> Physcomitrella patens

<400> 135
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 Gn Gy Val Asn Ala Leu Leu Gy Ser Phe Gy Val Gu Leu Thr Asp
 20 25 30
 Thr Pro Thr Thr Lys Gy Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
 35 40 45
 Val Leu Gy Val Ser Val Tyr Leu Thr Ile Val Ile Gy Gy Leu Leu
 50 55 60
 Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Gu Pro Phe Leu
 65 70 75 80
 Leu Gn Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
 85 90 95
 Leu Tyr Met Cys Val Gy Ile Ala Tyr Asn Pro Lys His Lys Gu Met Ala Ile
 100 105 110
 Ser Leu Trp Gy Asn Ala Tyr Asn Pro Lys Tyr Val Gu Met Asp Thr
 115 120 125
 Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Gu Phe Met Asp Thr
 130 135 140
 Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gn Ile Ser Phe Leu His
 145 150 155 160
 Val Tyr His His Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
 165 170 175
 His Ala Pro Gy Gy Gu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gy
 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 Gy Arg Tyr Leu
 210 215 220
 Thr Gn Phe Gn Met Phe Gn Phe Met Leu Asn Leu Val Gn Ala Tyr
 225 230 235 240
 Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gn Trp Leu Ile Lys Ile
 245 250 255
 Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gy Asn Phe Tyr
 260 265 270
 Val Gn Lys Tyr Ile Lys Pro Ser Asp Gy Lys Gn Lys Gy Ala Lys
 275 280 285
 Thr Gu
 290

<210> 136
 <211> 957
 <212> DNA
 <213> Mbrtierella alpina

<220>
 <221> CDS
 <222> (1)..(957)

<400> 136
 at g gag t cg att gcg cca ttc ct c cca t ca aag at g ccg caa gat ct g 48
 Met Gl u Ser lle Ala Pro Phe Leu Pro Ser Lys Met Pro Gl n Asp Leu
 1 5 10 15
 ttt atg gac ctt gcc acc gct at c ggt gt c cgg gcc gcg ccc t at gt c 96
 Phe Met Asp Leu Ala Thr Ala lle Gly Val Arg Ala Ala Pro Tyr Val
 20 25 30
 gat cct ct c gag gcc gcg ct g gt g gcc cag gcc gag aag t ac at c ccc 144
 Asp Pro Leu Gl u Ala Ala Leu Val Ala Gl n Ala Gl u Lys Tyr lle Pro
 35 40 45
 acg att gt c cat cac acg cgt ggg ttc ct g gt c gcg gt g gag t cg cct 192
 Thr lle Val His His Thr Arg Gly Phe Leu Val Ala Val Gl u Ser Pro
 50 55 60
 ttg gcc cgt gag ct g ccg tt g at g aac ccg ttc cac gt g ct g tt g at c 240
 Leu Ala Arg Gl u Leu Pro Leu Met Asn Pro Phe His Val Leu Leu lle
 65 70 75 80
 gt g ct c gct t at tt g gt c acg gt c ttt gt g ggc at g cag at c at g aag 288
 Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gl n lle Met Lys
 85 90 95
 aac ttt gag cgg ttc gag gt c aag acg ttt t cg ct c ct g cac aac ttt 336
 Asn Phe Gl u Arg Phe Gl u Val Lys Thr Phe Ser Leu Leu His Asn Phe
 100 105 110
 t gt ct g gt c t cg at c agc gcc t ac at g t gc ggt ggg at c ct g t ac gag 384
 Cys Leu Val Ser lle Ser Ala Tyr Met Cys Gly Gly lle Leu Tyr Gl u
 115 120 125
 gct t at cag gcc aac t at gga ct g ttt gag aac gct gct gat cat acc 432
 Ala Tyr Gl n Ala Asn Tyr Gly Leu Phe Gl u Asn Ala Ala Asp His Thr
 130 135 140
 ttc aag ggt ctt cct at g gcc aag at g at c tgg ct c ttc t ac ttc t cc 480
 Phe Lys Gly Leu Pro Met Ala Lys Met lle Trp Leu Phe Tyr Phe Ser
 145 150 155 160
 aag at c at g gag ttt gt c gac acc at g at c at g gt c ct c aag aag aac 528
 Lys lle Met Gl u Phe Val Asp Thr Met lle Met Val Leu Lys Lys Asn
 165 170 175
 aac cgc cag at c tcc ttc ttg cac gtt t ac cac cac agc tcc at c ttc 576
 Asn Arg Gl n lle Ser Phe Leu His Val Tyr His His Ser Ser lle Phe
 180 185 190
 acc at c tgg tgg ttg gt c acc ttt gt t gca ccc aac ggt gaa gcc t ac 624
 Thr lle Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Gl u Ala Tyr
 195 200 205
 ttc t ct gct gcg ttg aac t cg ttc at c cat gt g at c at g t ac ggc t ac 672
 Phe Ser Ala Ala Leu Asn Ser Phe lle His Val lle Met Tyr Gly Tyr
 210 215 220
 t ac ttc ttg t cg gcc ttg ggc ttc aag cag gt g t cg ttc at c aag ttc 720
 Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gl n Val Ser Phe lle Lys Phe
 225 230 235 240
 t ac at c acg cgc t cg cag at g aca cag ttc t gc at g at g t cg gt c cag 768
 Tyr lle Thr Arg Ser Gl n Met Thr Gl n Phe Cys Met Met Ser Val Gl n
 245 250 255
 t ct t cc tgg gac at g t ac gcc at g aag gt c ct t ggc cgc ccc gga t ac 816
 Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
 260 265 270
 ccc ttc ttc at c acg gct ct g ctt tgg ttc t ac at g tgg acc at g ct c 864
 Pro Phe Phe lle Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
 275 280 285
 ggt ct c ttc t ac aac ttt t ac 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 Gl n
 290 295 300
 gcc aag gcc gac gct gcc aag gag aag gca agg aag ttg cag t aa 957
 Ala Lys Ala Asp Ala Ala Lys Gl u Lys Ala Arg Lys Leu Gl n
 305 310 315

<210> 137
 <211> 318

<212> PRT

<213> Mbrtierella alpina

<400> 137

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Met 1  Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
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Phe 2  Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
20      25      30
Asp 3  Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
35      40      45
Thr 4  Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
50      55      60
Leu 5  Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65      70      75      80
Val 6  Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
85      90      95
Asn 7  Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
100     105
Cys 8  Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
115     120     125
Ala 9  Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
130     135     140
Phe 10 Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145     150     155     160
Lys 11 Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
165     170     175
Asn 12 Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
180     185     190
Thr 13 Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
195     200     205
Phe 14 Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
210     215     220
Tyr 15 Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225     230     235     240
Tyr 16 Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
245     250     255
Ser 17 Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
260     265     270
Pro 18 Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
275     280     285
Gly 19 Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
290     295     300
Ala 20 Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305     310     315

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<210> 138

<211> 242

<212> PRT

<213> Artificial sequence

<220>

<221> Variant

<222> (3)..(5)

<223> xaa in position 3 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)..(16)

<223> xaa in position 8 to 16 is any amino acid

<220>

<221> Variant

<222> (19)..(20)

<223> xaa in position 19 to 20 is any amino acid

<220>

<221> Variant
<222> (22)..(42)
<223> xaa in position 22 to 42 is any amino acid
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<221> Variant
<222> (43)..(49)
<223> xaa in position 43 to 49 is any or no amino acid
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<221> Variant
<222> (52)..(56)
<223> xaa in position 52 to 56 is any amino acid
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<221> Variant
<222> (59)..(59)
<223> xaa in position 59 is any amino acid
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<221> Variant
<222> (62)..(63)
<223> xaa in position 62 to 63 is any amino acid
<220>
<221> Variant
<222> (65)..(68)
<223> xaa in position 65 to 68 is any amino acid
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<221> Variant
<222> (70)..(73)
<223> xaa in position 70 to 73 is any amino acid
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<222> (75)..(75)
<223> xaa in position 75 is any amino acid
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<221> Variant
<222> (77)..(78)
<223> xaa in position 77 to 78 is any amino acid
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<221> Variant
<222> (80)..(94)
<223> xaa in position 80 to 94 is any amino acid
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<222> (95)..(98)
<223> xaa in position 95 to 98 is any or no amino acid
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<221> Variant
<222> (101)..(101)
<223> xaa in position 101 is any amino acid
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<221> Variant
<222> (104)..(105)
<223> xaa in position 104 to 105 is any amino acid
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<221> Variant
<222> (108)..(108)
<223> xaa in position 108 is any amino acid
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<221> Variant
<222> (111)..(111)
<223> xaa in position 111 is any amino acid
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<221> Variant
<222> (114)..(114)
<223> xaa in position 114 is any amino acid
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<221> Variant
<222> (116)..(120)
<223> xaa in position 116 to 120 is any amino acid
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<221> Variant
<222> (122).. (124)
<223> xaa in position 122 to 124 is any amino acid
<220>
<221> Variant
<222> (127).. (127)
<223> xaa in position 127 is any amino acid
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<221> Variant
<222> (131).. (132)
<223> xaa in position 131 to 132 is any amino acid
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<222> (134).. (135)
<223> xaa in position 134 to 135 is any amino acid
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<222> (139).. (143)
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<222> (148).. (149)
<223> xaa in position 148 to 149 is any amino acid
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<222> (151).. (151)
<223> xaa in position 151 is any amino acid
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<221> Variant
<222> (154).. (155)
<223> xaa in position 154 to 155 is any amino acid
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<222> (158).. (159)
<223> xaa in position 158 to 159 is any amino acid
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<221> Variant
<222> (162).. (162)
<223> xaa in position 162 is any amino acid
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<221> Variant
<222> (165).. (165)
<223> xaa in position 165 is any amino acid
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<221> Variant
<222> (168).. (168)
<223> xaa in position 168 is any amino acid
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<221> Variant
<222> (170).. (184)
<223> xaa in position 170 to 184 is any amino acid
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<221> Variant
<222> (185).. (190)
<223> xaa in position 185 to 190 is any or no amino acid
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<221> Variant
<222> (192).. (193)
<223> xaa in position 192 to 193 is any amino acid
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<221> Variant
<222> (196).. (196)
<223> xaa in position 196 is any amino acid
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<221> Variant
<222> (199).. (203)
<223> xaa in position 199 to 203 is any amino acid
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<221> Variant
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<223> xaa in position 205 to 206 is any amino acid
<220>
<221> Variant
<222> (208)..(213)
<223> xaa in position 208 to 213 is any amino acid
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<221> Variant
<222> (214)..(219)
<223> xaa in position 214 to 219 is any or no amino acid
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<221> Variant
<222> (222)..(227)
<223> xaa in position 222 to 227 is any amino acid
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<221> Variant
<222> (229)..(230)
<223> xaa in position 229 to 230 is any amino acid
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<221> Variant
<222> (233)..(235)
<223> xaa in position 233 to 235 is any amino acid
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<221> Variant
<222> (237)..(237)
<223> xaa in position 237 is any amino acid
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<221> Variant
<222> (241)..(241)
<223> xaa in position 241 is any amino acid

<400> 138
Leu Pro xaa xaa xaa xaa Pro xaa xaa xaa xaa xaa xaa xaa xaa xaa
  1      5      10      15
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 Gu Phe xaa Asp Thr xaa Ile
  100     105     110
Met xaa Leu xaa xaa xaa xaa xaa xaa Gn xaa xaa xaa Leu His xaa Tyr
  115     120     125
His His xaa xaa Ile xaa xaa Ile Trp Trp 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 xaa Thr xaa
  180     185     190
xaa Gn Met xaa Gn Phe xaa xaa xaa xaa xaa Gn xaa xaa Tyr xaa
  195     200     205
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

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<210> 139

<211> 60
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Phe or Trp
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is Leu, Met or Val
<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 Phe or Ile
<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)..(15)
<223> xaa in position 13 to 15 is any amino acid
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is any or no amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Ile, Leu or Val
<220>
<221> Variant
<222> (19)..(19)
<223> xaa in position 19 is Ser or Thr
<220>
<221> Variant
<222> (20)..(20)
<223> xaa in position 20 is Phe or Val
<220>
<221> Variant
<222> (23)..(23)
<223> xaa in position 23 is Ile or Val
<220>
<221> Variant
<222> (27)..(27)
<223> xaa in position 27 is Ala, Ser or Thr
<220>
<221> Variant
<222> (28)..(28)
<223> xaa in position 28 is Ser or Thr
<220>
<221> Variant
<222> (29)..(29)
<223> xaa in position 29 is Ile or Val
<220>
<221> Variant
<222> (30)..(31)
<223> xaa in position 30 to 31 is any amino acid

<220>
 <221> Vari ant
 <222> (32) .. (32)
 <223> xaa in position 32 is Ile or Leu
 <220>
 <221> Vari ant
 <222> (34) .. (34)
 <223> xaa in position 34 is any ami no aci d
 <220>
 <221> Vari ant
 <222> (35) .. (35)
 <223> xaa in position 35 is Ala, Ile or Leu
 <220>
 <221> Vari ant
 <222> (36) .. (36)
 <223> xaa in position 36 is Ala, Ile or Val
 <220>
 <221> Vari ant
 <222> (37) .. (39)
 <223> xaa in position 37 to 39 is any ami no aci d
 <220>
 <221> Vari ant
 <222> (42) .. (42)
 <223> xaa in position 42 is Gly or Asn
 <220>
 <221> Vari ant
 <222> (44) .. (44)
 <223> xaa in position 44 is Asp or Glu
 <220>
 <221> Vari ant
 <222> (45) .. (45)
 <223> xaa in position 45 is Ala or Ser
 <220>
 <221> Vari ant
 <222> (47) .. (47)
 <223> xaa in position 47 is Phe, Trp or Tyr
 <220>
 <221> Vari ant
 <222> (49) .. (49)
 <223> xaa in position 49 is Ala or Thr
 <220>
 <221> Vari ant
 <222> (50) .. (50)
 <223> xaa in position 50 is any ami no aci d
 <220>
 <221> Vari ant
 <222> (51) .. (51)
 <223> xaa in position 51 is Leu or Val
 <220>
 <221> Vari ant
 <222> (54) .. (54)
 <223> xaa in position 54 is any ami no aci d
 <220>
 <221> Vari ant
 <222> (55) .. (55)
 <223> xaa in position 55 is Ile or Val
 <220>
 <221> Vari ant
 <222> (58) .. (58)
 <223> xaa in position 58 is any ami no aci d
 <220>
 <221> Vari ant
 <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

PF58307.txt

G n xaa xaa xaa Leu H i s xaa Tyr H i s H i s xaa 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 H i s Val xaa xaa Tyr
 50 55 60

<210> 140
<211> 22
<212> PRT
<213> Artificial sequence

<220>
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<222> (2)..(2)
<223> xaa in position 2 is Ala or Leu
<220>
<221> Variant
<222> (3)..(3)
<223> xaa in position 3 is Phe or Tyr
<220>
<221> Variant
<222> (5)..(5)
<223> xaa in position 5 is Cys or Ser
<220>
<221> Variant
<222> (6)..(6)
<223> xaa in position 6 is Gly, Leu or Val
<220>
<221> Variant
<222> (8)..(9)
<223> xaa in position 8 to 9 is any amino acid
<220>
<221> Variant
<222> (10)..(11)
<223> xaa in position 10 to 11 is any or no amino acid
<220>
<221> Variant
<222> (13)..(14)
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<221> Variant
<222> (15)..(16)
<223> xaa in position 15 to 16 is any or no amino acid
<220>
<221> Variant
<222> (18)..(18)
<223> xaa in position 18 is Gly, Ser or Thr
<220>
<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 Gu, 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>
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 <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
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 <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
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<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
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 <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 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 Gu 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

PF58307.txt

I l e A r g G l u P r o T h r T r p L e u A r g A r g P h e I l e I l e C y s H i s A s n A l a
85 90 95
t t c t t g a t a g t c c t c a g t c t t t a c a t g t g c c t t g g t t g t g t g g c c c a a
P h e L e u I l e V a l L e u S e r L e u T y r M e t C y s L e u G l y C y s V a l A l a G l n 336
100
g c g t a t c a g a a t g g a t a t a c t t t a t g g g g t a a t g a a t t t c a a g g c c a c g
A l a T y r G l n A s n G l y T y r T h r L e u T r p G l y A s n G l u P h e L y s A l a T h r 384
115
g a a a c t c a g c t t g c t c t a c a t t t a c a t t t t t t a c g t a a g t a a a a t a
G u T h r G l n L e u A l a L e u T y r I l e T y r I l e P h e T y r V a l S e r L y s I l e 432
130
t a c g a g t t t g t a g a t a c t t a c a t t a t g c t t c t c a a g a a t a a c t t g c g g
T y r G l u P h e V a l A s p T h r T y r I l e M e t L e u L e u L y s A s n A s n L e u A r g
145 150 155 160
c a a g t a a g t t t c c t a c a c a t t t a t c a c c a c a g c a c g a t t t c c t t t a t t
G n V a l S e r P h e L e u H i s I l e T y r H i s H i s S e r T h r I l e S e r P h e I l e 528
165 170 175
t g g t g g a t c a t t g c t c g g a g g g c t c c g g g t g a t g c t t a c t t c a g c
T r p T r p I l e I l e A l a A r g A r g A l a P r o G l y G l y A s p A l a T y r P h e S e r
180 185
g c g g c c t t g a a c t c a t g g g t a c a c g t g t g c a t g t a c a c c t a t t a t c t a
A l a A l a L e u A s n S e r T r p V a l H i s V a l C y s M e t T y r T h r T y r T y r L e u 624
195
t t a t c a a c c c t t a t t g g a a a g a a g a t c c t a a g c g t t c c a a c t a c c t t
L e u S e r T h r L e u I l e G l y L y s G l u A s p P r o L y s A r g S e r A s n T y r L e u 672
210 215 220
t g g t g g g g t c g c c a c c t a a c g c a a a t g c a g a t g c t t c a g t t t t t c t t c
T r p T r p G l y A r g H i s L e u T h r G n M e t G n M e t L e u G n P h e P h e P h e 720
225 230 235 240
a a c g t a c t t c a a g c g t t g t a c t g c g c t t c g t t c t c t a c g t a t c c c a a g
A s n V a l L e u G n A l a L e u T y r C y s A l a S e r P h e S e r T h r T y r P r o P r o L y s
245 250 255
t t t t t g t c c a a a a t t c t g c t c g t c t a t a t g a t g a g c c t t c t c g g c t t g
P h e L e u S e r L y s I l e L e u L e u V a l T y r M e t M e t S e r L e u L e u G l y L e u 816
260 265 270
t t t g g g c a t t t c t a c t a t t c c a a g c a c a t a g c a g c a g t a a g c t c c a g
P h e G l y H i s P h e T y r T y r S e r L y s H i s I l e A l a A l a A l a L y s L e u G n 864
275 280 285
a a a a a c a g c a g t g a
L y s L y s G n G n 879
290

<210> 144
<211> 292
<212> PRT
<213> *Ostreococcus tauri*

<400> 144
M e t S e r G l y L e u A r g A l a P r o A s n P h e L e u H i s A r g P h e T r p T h r L y s
1 5 10 15
T r p A s p T y r A l a I l e S e r L y s V a l V a l P h e T h r C y s A l a A s p S e r P h e
20 25 30
G n T r p A s p I l e G l y P r o V a l S e r S e r S e r T h r A l a H i s L e u P r o A l a
35 40 45
I l e G l u S e r P r o T h r P r o L e u V a l T h r S e r L e u L e u P h e T y r L e u V a l
50 55 60
T h r V a l P h e L e u T r p T y r G l y A r g L e u T h r A r g S e r S e r A s p L y s L y s
65 70 75 80
I l e A r g G l u P r o T h r T r p L e u A r g A r g P h e I l e I l e C y s H i s A s n A l a
85 90 95
P h e L e u I l e V a l L e u S e r L e u T y r M e t C y s L e u G l y C y s V a l A l a G n
100 105 110
A l a T y r G l n A s n G l y T y r T h r L e u T r p G l y A s n G l u P h e L y s A l a T h r
115 120 125
G u T h r G l n L e u A l a L e u T y r I l e T y r I l e P h e T y r V a l S e r L y s I l e
130 135 140
T y r G l u P h e V a l A s p T h r T y r I l e M e t L e u L e u L y s A s n A s n L e u A r g
145 150 155 160

PF58307. txt

G n Val Ser Phe Leu H i s I l e Tyr H i s H i s Ser Thr I l e Ser Phe I l e
 165 170 175
 Tr p Tr p I l e I l e A l a Arg Arg A l a Pro G y G y Asp A l a Tyr Phe Ser
 180 185
 A l a A l a Leu Asn Ser Tr p Val H i s Cys Met Tyr Thr Tyr Tyr Leu
 195 200 205
 Leu Ser Thr Leu I l e G y Lys G u Asp Pro Lys Arg Ser Asn Tyr Leu
 210 215 220
 Tr p Tr p G y Arg H i s Leu Thr G n Met G n Met Leu G n Phe Phe Phe
 225 230 235
 Asn Val Leu G n A l a Leu Tyr Cys A l a Ser Phe Ser Thr Tyr Pro Lys
 245 250 255
 Phe Leu Ser Lys I l e Leu Leu Val Tyr Met Met Ser Leu Leu G y Leu
 260 265 270
 Phe G y H i s Phe Tyr Tyr Ser Lys H i s I l e A l a A l a A l a Lys Leu G n
 275 280 285
 Lys Lys G n G n
 290

<210> 145
 <211> 831
 <212> DNA
 <213> Thr aust ochyt r i um sp

<220>
 <221> CDS
 <222> (1) . . (831)

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 at g gat gt c gt c gag cag caa tgg cgc cgc t t c gt g gac gcc gt g gac 48
 Met Asp Val Val G u G n G n Tr p Arg Arg Phe Val Asp A l a Val Asp
 1 5 10 15
 aac gga at c gt g gag t t c at g gag cat gag gag ccc aac aag ct g aac 96
 Asn G y I l e Val G u Phe Met G u H i s G u G u Pro Asn Lys Leu Asn
 20 25 30
 gag ggc aag ct c t cc acc t cg acc gag gag at g at g gcg ct t at c gt c 144
 G u G y Lys Leu Ser Thr Ser Thr G u G u Met Met A l a Leu I l e Val
 35 40 45
 ggc t ac ct g gcg t t c gt g gt c ct c ggg t cc gcc t t c at g aag gcc t t t 192
 G y Tyr Leu A l a Phe Val Val Leu G y Ser A l a Phe Met Lys A l a Phe
 50 55 60
 gt c gat aag cct t t c gag ct c aag t t c ct c aag ct c gt g cac aac at c 240
 Val Asp Lys Pro Phe G u Leu Lys Phe Leu Lys Leu Val H i s Asn I l e
 65 70 75 80
 t t c ct c acc ggt ct g t cc at g t ac at g gcc acc gag t gc gcg cgc cag 288
 Phe Leu Thr G y Leu Ser Met Tyr Met A l a Thr G u Cys A l a Arg G n
 85 90 95
 gca t ac ct c ggc ggc t ac aag ct c t t t ggc aac ccg at g gag aag ggc 336
 A l a Tyr Leu G y G y Tyr Lys Leu Phe G y Asn Pro Met G u Lys G y
 100 105 110
 acc gag t cg cac gcc ccg ggc at g gcc aac at c at c t ac at c t t c t ac 384
 Thr G u Ser H i s A l a Pro G y Met A l a Asn I l e I l e Tyr I l e Phe Tyr
 115 120 125
 gt g agc aag t t c ct c gaa t t c ct c gac acc gt c t t c at g at c ct c ggc 432
 Val Ser Lys Phe Leu G u Phe Leu Asp Thr Val Phe Met I l e Leu G y
 130 135 140
 aag aag tgg aag cag ct c agc t t t ct c cac gt c t ac cac cac gcg agc 480
 Lys Lys Tr p Lys G n Leu Ser Phe Leu H i s Val Tyr H i s H i s A l a Ser
 145 150 155 160
 at c agc t t c at c tgg ggc at c at c gcc cgc t t c gcg ccc ggt ggc gac 528
 I l e Ser Phe I l e Tr p G y I l e I l e A l a Arg Phe A l a Pro G y G y Asp
 165 170 175
 gcc t ac t t c t ct acc at c ct c aac agc agc gt g cat gt c gt g ct c t ac 576
 A l a Tyr Phe Ser Thr I l e Leu Asn Ser Ser Val H i s Val Val Leu Tyr
 180 185 190
 ggc t ac t ac gcc t cg acc acc ct c ggc t ac acc t t c at g cgc ccg ct g 624
 G y Tyr Tyr A l a Ser Thr Thr Leu G y Tyr Thr Phe Met Arg Pro Leu
 195 200 205

PF58307. txt

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	
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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	
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Gln	Lys	Thr	Ile													
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			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		
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Val	Asp	Lys	Pro	Phe	Glu	Leu	Lys	Phe	Leu	Lys	Leu	Val	His	Asn	Ile		
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Phe	Leu	Thr	Gly	Leu	Ser	Met	Tyr	Met	Ala	Thr	Glu	Cys	Ala	Arg	Gln		
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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		
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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		
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Gln	Lys	Thr	Ile														
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<220>
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PF58307. txt

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cct gac tgg gt c ttg aca aag t ct gcg gcg ct g gt g t ac agc t gc t t c 96
Pro Asp Trp Val Leu Thr Lys Ser Ala Ala Leu Val Tyr Ser Cys Phe
20 25 30
ggc t cc gcc gcc aat gct t t t gaa agc agt at c aag at c aac t t c ccg 144
G y Ser Ala Ala Asn Ala Phe Gu Ser Ser Ile Lys Ile Asn Phe Pro
35 40 45
gcg cag cat gcg t t c gt g gag gcg t gg at g cgc gcg cgc t cc cac cct 192
Ala Gn His Ala Phe Val Gu Ala Trp Met Arg Ala Arg Ser His Pro
50 55 60
t t t gcg gag cgc ct g ccg t ac ct g aat ccg t gg cac gt t at c gcc t cg 240
Phe Ala Gu Arg Leu Pro Tyr Leu Asn Pro Trp His Val Ile Ala Ser
65 70 75 80
at a ct g gcc t ac ct c t cc t t g att gt c acc t t g cgc ct g t t g cat cgt 288
Ile Leu Ala Tyr Leu Ser Leu Ile Val Thr Leu Arg Leu Leu His Arg
85 90 95
gt a ct c ggt aag t t c t cg t gc cgc act ct c gga t t g gt g cac aac ct c 336
Val Leu G y Lys Phe Ser Cys Arg Thr Leu G y Leu Val His Asn Leu
100 105
ggt ct c cat ct t ct c t cg t t g t ac at g agc ct t ggt ct c at g at c agc 384
G y Leu His Leu Leu Ser Leu Tyr Met Ser Leu G y Leu Met Ile Ser
115 120 125
gcg cgc gcc gcg ggg t ac t cg ct c t gg aac aac gcg gt c ggc acc t cc 432
Ala Arg Ala Ala G y Tyr Ser Leu Trp Asn Asn Ala Val G y Thr Ser
130 135 140
ccg gct gag t gg cgc att gcg aag ct g at c t gg ct c t t c t at gt c t cg 480
Pro Ala Gu Trp Arg Ile Ala Lys Leu Ile Trp Leu Phe Tyr Val Ser
145 150 155 160
aag gt g gt g gaa t gg gt g gac acg gt a at t at g t t a t t a aag cag aac 528
Lys Val Val Gu Trp Val Asp Thr Val Ile Met Leu Leu Lys G n Asn
165 170 175 180
t ac cac cag gt c acc t t c ct g cac gt g t at cac cac acg acg gt t t t t 576
Tyr His Gn Val Thr Phe Leu His Val Tyr His His Thr Thr Val Phe
185 190
gt g ct g t gg t gg ct g gcg t t g ct g gt c gct cct ggc ggc gag t cg t ac 624
Val Leu Trp Trp Leu Ala Leu Leu Val Ala Pro G y G y G u Ser Tyr
195 200 205
t ac agc gcc at g gt g aac t ct ggc gt c cac gt t t t c at g t ac ggg t ac 672
Tyr Ser Ala Met Val Asn Ser G y Val His Val Phe Met Tyr G y Tyr
210 215 220
t ac t t t ct c acg ct g ct c t t c cca t cc ggc at c gt g cgc gac gt c t t g 720
Tyr Phe Leu Thr Leu Leu Phe Pro Ser G y Ile Val Arg Asp Val Leu
225 230 235 240
agc aag t t c aag t t t gcc att acg aag ggc cag at g t gg cag t t c gt c 768
Ser Lys Phe Lys Phe Ala Ile Thr Lys G y Gn Met Trp Gn Phe Val
245 250 255
t t c aac t gc ct a cag t cc gcg t ac gac ct c gt g t gg gt g ccg ccg gaa 816
Phe Asn Cys Leu Gn Ser Ala Tyr Asp Leu Val Trp Val Pro Arg Gu
260 265 270 275
gag ct c aag t ac agc gcg gag ct g ct g cag at c ct c t t c t gg t ac at g 864
Gu Leu Lys Tyr Ser Ala Gu Leu Leu Gn Ile Leu Phe Trp Tyr Met
280 285
at c t cc ct c t t g gcg ct c t t t ggc aac t t c t t g gt g aag aac aag aag 912
Ile Ser Leu Leu Ala Leu Phe G y Asn Phe Leu Val Lys Asn Lys Lys
290 295 300
t t c t cg cac cgc cgc t gc gt t gat gcc gcg act gct t cg ggc gcg aag 960
Phe Ser His Arg Arg Cys Val Asp Ala Ala Thr Ala Ser G y Ala Lys
305 310 315 320
gag gac acg gcg gcg agg t cc cac ggc gac cgc acc cac aga acc cgt 1008
Gu Asp Thr Ala Ala Arg Ser His G y Asp Arg Thr His Arg Thr Arg
325 330 335
gt g aag gct ggc at g acc aac at g caa ct g gag agg ct g aag aat gag 1056
Val Lys Ala G y Met Thr Asn Met Gn Leu Gu Arg Leu Lys Asn Gu
340 345 350
aag t cc acg gag at g aag ct g ct g at g cgc aag aac ggc aac ggc aac 1104

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Lys Ser Thr Gu Met Lys Leu Leu Met Arg Lys Asn Gly Asn Gly Asn
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 gga caa aaa gcg t cg ct c cag gcc at g gca ggc agt cga t ga
 Gly Gn Lys Ala Ser Leu Gn Ala Met Ala Gly Ser Arg
 370 375 380

1146

<210> 148
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 <212> PRT
 <213> Lei shmani a maj or

<400> 148
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 Pro Asp Trp Val Leu Thr Lys Ser Ala Ala Leu Val Tyr Ser Cys Phe
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 Gly Ser Ala Ala Asn Ala Phe Gu Ser Ser Ile Lys Ile Asn Phe Pro
 35 40 45
 Ala Gn His Ala Phe Val Gu Ala Trp Met Arg Ala Arg Ser His Pro
 50 55 60
 Phe Ala Gu Arg Leu Pro Tyr Leu Asn Pro Trp His Val Ile Ala Ser
 65 70 75
 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 Gu Trp Arg Ile Ala Lys Leu Ile Trp Leu Phe Tyr Val Ser
 145 150 155
 Lys Val Val Gu Trp Val Asp Thr Val Ile Met Leu Leu Lys Gn Asn
 165 170 175
 Tyr His Gn 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 Gu 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
 Ser Lys Phe Lys Phe Ala Ile Thr Lys Gly Gn Met Trp Gn Phe Val
 245 250 255
 Phe Asn Cys Leu Gn Ser Ala Tyr Asp Leu Val Trp Val Pro Arg Gu
 260 265 270
 Gu Leu Lys Tyr Ser Ala Gu Leu Leu Gn 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
 Gu 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 Gn Leu Gu Arg Leu Lys Asn Gu
 340 345 350
 Lys Ser Thr Gu Met Lys Leu Leu Met Arg Lys Asn Gly Asn Gly Asn
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 Gly Gn Lys Ala Ser Leu Gn Ala Met Ala Gly Ser Arg
 370 375 380

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 <212> DNA
 <213> Ostreococcus tauri

<220>
 <221> CDS

<222> (1)..(879)

<400> 149

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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	
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aca	ggt	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	gat	gct	tac	ttc	agg	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	atg	gca	gca	gct	aag	ctc	cag	864
Phe	Gly	His	Phe	Tyr	Tyr	Ser	Lys	His	Ile	Ala	Ala	Ala	Lys	Leu	Gln	
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aaa	aaa	cag	cag	tga												879
Lys	Lys	Gln	Gln													
			290													

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<211> 292

<212> PRT

<213> *Ostreococcus tauri*

<400> 150

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PF58307. txt

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 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 Gu 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 Gn
 100 105 110
 Ala Tyr Gn Asn Gly Tyr Thr Leu Trp Gly Asn Gu Phe Lys Ala Thr
 115 120 125
 Gu Thr Gn Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
 130 135 140
 Tyr Gu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
 145 150 155 160
 Gn 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 Gu Asp Pro Lys Arg Ser Asn Tyr Leu
 210 215 220
 Trp Trp Gly Arg His Leu Thr Gn Met Gn Met Leu Gn Phe Phe Phe
 225 230 235 240
 Asn Val Leu Gn 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
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 Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gn
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 Lys Lys Gn Gn
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 Gn Gy Val Asn Ala Leu Leu Gy Ser Phe Gy Val Gu Leu Thr Asp
 20 25 30
 acg ccc act acc aaa ggc ttg ccc ctg gtt gac agt ccc aca ccc atc 144
 Thr Pro Thr Thr Lys Gy Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
 35 40 45
 gtc ctg ggt gtt tct gta tac ttg act att gtc att gga ggg ctt ttg 192
 Val Leu Gy Val Ser Val Tyr Leu Thr Ile Val Ile Gy Gy 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 Gu Pro Phe Leu
 65 70 75 80
 ctg caa gct ttg gtg ctt gtg cac aac ctg ttc tgt ttt gcg ctg agt 288
 Leu Gn Ala Leu Val 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 Gy Ile Ala Tyr Gn Ala Ile Thr Trp Arg Tyr

PF58307.txt

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Ser	Leu	Tr p	G y	Asn	Al a	Tyr	Asn	Pro	Lys	Hi s	Lys	G u	M et	Al a	I le				
		115						120											
ct g	gt a	t ac	t tg	t tc	t ac	at g	t ct	a ag	t ac	gt g	gaa	t tc	at g	gat	acc				432
Leu	Val	Tyr	Leu	Phe	Tyr	M et	Ser	Lys	Tyr	Val	G u	Phe	M et	Asp	Thr				
		130						135											
gt t	at c	at g	at a	ct g	a ag	cg c	ag c	acc	agg	caa	at a	ag c	t tc	ct c	cac				480
Val	I le	M et	I le	Leu	Lys	Arg	Ser	Thr	Arg	G n	I le	Ser	Phe	Leu	Hi s				160
		145						150											
gt t	t at	cat	cat	t ct	t ca	at t	t cc	ct c	at t	t gg	t gg	gct	at t	gct	cat				528
Val	Tyr	Hi s	Hi s	Ser	Ser	I le	Ser	Leu	I le	Tr p	Tr p	Al a	I le	Al a	Hi s				
		165						170											
cac	gct	c ct	ggc	ggt	gaa	gca	t at	t gg	t ct	gcg	gct	ct g	aac	t ca	gga				576
Hi s	Al a	Pro	G y	G y	G u	Al a	Tyr	Tr p	Ser	Al a	Al a	Leu	Asn	Ser	G y				
		180						185											
gt g	cat	gt t	ct c	at g	t at	gcg	t at	t ac	t tc	t tg	gct	gcc	t gc	ct t	cga				624
Val	Hi s	Val	Leu	M et	Tyr	Al a	Tyr	Tyr	Phe	Leu	Al a	Al a	O ys	Leu	Arg				
		195						200											
agt	agc	cca	a ag	t ta	aaa	aat	a ag	t ac	ct t	t tt	t gg	ggc	agg	t ac	t tg				672
Ser	Ser	Pro	Lys	Leu	Lys	Asn	Lys	Tyr	Leu	Phe	Tr p	G y	Arg	Tyr	Leu				
		210						215											
aca	caa	t tc	caa	at g	t tc	cag	t tt	at g	ct g	aac	t ta	gt g	cag	gct	t ac				720
Thr	G n	Phe	G n	M et	Phe	G n	Phe	M et	Leu	Asn	Leu	Val	G n	Al a	Tyr				240
		225						230											
t ac	gac	at g	aaa	acg	aat	gcg	cca	t at	cca	caa	t gg	ct g	at c	aag	at t				768
Tyr	Asp	M et	Lys	Thr	Asn	Al a	Pro	Tyr	Pro	G n	Tr p	Leu	I le	Lys	I le				
		245						250											
t tg	t tc	t ac	t ac	at g	at c	t cg	t tg	ct g	t tt	ct t	t tc	ggc	aat	t tt	t ac				816
Leu	Phe	Tyr	Tyr	M et	I le	Ser	Leu	Leu	Phe	Leu	Phe	G y	Asn	Phe	Tyr				
		260						265											
gt a	caa	aaa	t ac	at c	aaa	ccc	t ct	gac	gga	a ag	caa	a ag	gga	gct	aaa				864
Val	G n	Lys	Tyr	I le	Lys	Pro	Ser	Asp	G y	Lys	G n	Lys	G y	Al a	Lys				
		275						280											
act	gag	t ga																	873
Thr	G u																		
		290																	

<210> 152
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 <213> Physcomitrella patens

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 20 25 30
 Thr Pro Thr Thr Lys G y Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
 35 40 45
 Val Leu Thr G y Val Ser Val Tyr Leu Thr Ile Val Ile G y G y Leu Leu
 50 55 60
 Tr p Ile Lys Al a Arg Asp Leu Lys Pro Arg Al a Ser G u Pro Phe Leu
 65 70 75 80
 Leu G n Al a Leu Val Leu Val Hi s Asn Leu Phe Cys Phe Al a Leu Ser
 85 90 95
 Leu Tyr M et Cys Val G y Ile Al a Tyr G n Al a Ile Thr Tr p Arg Tyr
 100 105 110
 Ser Leu Tr p G y Asn Al a Tyr Asn Pro Lys Hi s Lys G u M et Al a Ile
 115 120 125
 Leu Val Tyr Leu Phe Tyr M et Ser Lys Tyr Val G u Phe M et Asp Thr
 130 135 140
 Val Ile M et Ile Leu Lys Arg Ser Thr Arg G n Ile Ser Phe Leu Hi s
 145 150 155 160
 Val Tyr Hi s Hi s Ser Ser Ile Ser Leu Ile Tr p Tr p Al a Ile Al a Hi s
 165 170 175
 Hi s Al a Pro G y G y G u Al a Tyr Tr p Ser Al a Al a Leu Asn Ser G y
 180 185 190

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Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
 Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
 Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
 Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
 Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
 Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
 Thr Gu
 290

<210> 153
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 Met Gu 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 ct c gag gcc gcg ct ggt gcc cag gcc gag aag tac atc ccc 144
 Asp Pro Leu Gu Ala Ala Leu Val Ala Gln Ala Gu Lys Tyr Ile Pro
 35 40 45
 acg att gtc cat cac acg cgt ggg ttc ct ggt gtc gcg gtt gag tgc cct 192
 Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Gu Ser Pro
 50 55 60
 ttg gcc cgt gag ct g ccg ttg atg aac ccg ttc cac gtt ct g ttg atc 240
 Leu Ala Arg Gu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
 65 70 75 80
 gtg ct c gct tat ttg gtc acg gtc ttt gtt 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 ccg ttc gag gtc aag acg ttt tgc ct c ct g cac aac ttt 336
 Asn Phe Gu Arg Phe Gu Val Lys Thr Phe Ser Leu Leu His Asn Phe
 100 105 110
 tgt ct g gtc tgc atc agc gcc tac atg tgc ggt ggg atc ct g tac gag 384
 Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Gu
 115 120 125
 gct tat cag gcc aac tat gga ct g ttt gag aac gct gct gat cat acc 432
 Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Gu Asn Ala Ala Asp His Thr
 130 135 140
 ttc aag ggt ctt cct atg gcc aag atg atc tgg ct c 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 ct c aag aag aac 528
 Lys Ile Met Gu 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 Gu Ala Tyr
 195 200 205
 ttc tct gct gcg ttg aac tgc ttc atc cat gtt 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 tgc gcc ttg ggc ttc aag cag gtt gtc ttc atc aag ttc 720

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Tyr 225	Phe	Leu	Ser	Ala	Leu 230	Gly	Phe	Lys	Gln	Val 235	Ser	Phe	Ile	Lys	Phe 240	
tac	atc	acg	cgc	tcg	cag	atg	aca	cag	ttc	tgc	atg	atg	tcg	gtc	cag	768
Tyr	Ile	Thr	Arg	Ser 245	Gln	Met	Thr	Gln	Phe 250	Cys	Met	Met	Ser	Val 255	Gln	
tct	tcc	tgg	gac	atg	tac	gcc	atg	aag	gtc	ctt	ggc	cgc	ccc	gga	tac	816
Ser	Ser	Trp	Asp 260	Met	Tyr	Ala	Met	Lys 265	Val	Leu	Gly	Arg	Pro 270	Gly	Tyr	
ccc	ttc	ttc	atc	acg	gct	ctg	ctt	tgg	ttc	tac	atg	tgg	acc	atg	ctc	864
Pro	Phe	Phe 275	Ile	Thr	Ala	Leu	Leu 280	Trp	Phe	Tyr	Met	Trp 285	Thr	Met	Leu	
ggt	ctc	ttc	tac	aac	ttt	tac	aga	aag	aac	gcc	aag	ttg	gcc	aag	cag	912
Gly	Leu 290	Phe	Tyr	Asn	Phe 295	Tyr	Arg	Lys	Asn	Ala 300	Lys	Leu	Ala	Lys	Gln	
gcc	aag	gcc	gac	gct	gcc	aag	gag	aag	gca	agg	aag	ttg	cag	t aa		957
Ala 305	Lys	Ala	Asp	Ala 310	Ala	Lys	Gu	Lys	Ala	Arg 315	Lys	Leu	Gln			

<210> 154

<211> 318

<212> PRT

<213> Mbrt ierel l a al pi na

<400> 154

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Phe	Met	Asp	Leu 20	Ala	Thr	Ala	Ile	Gly 25	Val	Arg	Ala	Ala	Pro 30	Tyr	Val	
Asp	Pro	Leu 35	Gu	Ala	Ala	Leu	Val 40	Ala	Gln	Ala	Gu	Lys 45	Tyr	Ile	Pro	
Thr	Ile 50	Val	His	His	Thr	Arg 55	Gly	Phe	Leu	Val	Ala 60	Val	Gu	Ser	Pro	
Leu 65	Ala	Arg	Gu	Leu 70	Pro	Leu	Met	Asn	Pro	Phe 75	His	Val	Leu	Leu 80	Ile	
Val	Leu	Ala	Tyr 85	Leu	Val	Thr	Val	Phe 90	Val	Gly	Met	Gln	Ile	Met 95	Lys	
Asn	Phe	Gu	Arg 100	Phe	Gu	Val	Lys	Thr 105	Phe	Ser	Leu	Leu	His 110	Asn	Phe	
Cys	Leu	Val 115	Ser	Ile	Ser	Ala	Tyr 120	Met	Cys	Gly	Gly	Ile 125	Leu	Tyr	Gu	
Ala	Tyr 130	Gln	Ala	Asn	Tyr	Gly 135	Leu	Phe	Gu	Asn 140	Ala	Ala	Asp	His	Thr	
Phe 145	Lys	Gly	Leu	Pro	Met 150	Ala	Lys	Met	Ile	Trp 155	Leu	Phe	Tyr	Phe	Ser 160	
Lys	Ile	Met	Gu 165	Val	Asp	Thr	Met	Ile 170	Met	Val	Leu	Lys	Lys	Asn 175		
Asn	Arg	Gln	Ile 180	Ser	Phe	Leu	His 185	Val	Tyr	His	His	Ser	Ser 190	Ile	Phe	
Thr	Ile	Trp 195	Trp	Leu	Val	Thr	Phe 200	Val	Ala	Pro	Asn	Gly 205	Gu	Ala	Tyr	
Phe	Ser 210	Ala	Ala	Leu	Asn	Ser 215	Phe	Ile	His	Val	Ile 220	Met	Tyr	Gly	Tyr	
Tyr 225	Phe	Leu	Ser	Ala	Leu 230	Gly	Phe	Lys	Gln	Val 235	Ser	Phe	Ile	Lys	Phe 240	
Tyr	Ile	Thr	Arg	Ser 245	Gln	Met	Thr	Gln	Phe 250	Cys	Met	Met	Ser	Val 255	Gln	
Ser	Ser	Trp	Asp 260	Met	Tyr	Ala	Met	Lys 265	Val	Leu	Gly	Arg	Pro 270	Gly	Tyr	
Pro	Phe	Phe 275	Ile	Thr	Ala	Leu	Leu 280	Trp	Phe	Tyr	Met	Trp 285	Thr	Met	Leu	
Gly	Leu 290	Phe	Tyr	Asn	Phe 295	Tyr	Arg	Lys	Asn	Ala	Lys 300	Leu	Ala	Lys	Gln	
Ala 305	Lys	Ala	Asp	Ala 310	Ala	Lys	Gu	Lys	Ala	Arg 315	Lys	Leu	Gln			

<210> 155

<211> 236

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<223> xaa in position 53 is any amino acid

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<223> xaa in position 69 is any amino acid

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<222> (71)..(72)
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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 121 is any amino acid
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<223> xaa in position 142 is any amino acid
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<223> xaa in position 186 to 187 is any amino acid
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<222> (193)..(197)
<223> xaa in position 193 to 197 is any amino acid
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<223> xaa in position 199 to 200 is any amino acid
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<222> (202)..(207)
<223> xaa in position 202 to 207 is any amino acid
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<222> (208)..(213)
<223> xaa in position 208 to 213 is any or no amino acid
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<222> (223)..(224)
<223> xaa in position 223 to 224 is any amino acid
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<222> (227)..(229)
<223> xaa in position 227 to 229 is any amino acid
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<223> xaa in position 235 is any amino acid

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Pro xaa xaa xaa xaa xaa xaa xaa Leu xaa Tyr Leu xaa xaa Val xaa
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 20      25      30
xaa 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 Ala xaa
 50      55
xaa xaa xaa Tyr xaa Leu xaa xaa Asn 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
xaa xaa Glu xaa Ser Phe Leu His xaa Tyr His His xaa xaa Ile xaa
 115
xaa Ile Trp Trp xaa xaa xaa xaa xaa Ala Pro Gly Gly xaa Ala Tyr
 130
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
 165      170      175
xaa xaa xaa xaa xaa xaa xaa xaa Thr xaa xaa Glu Met xaa Glu Phe
 180      185      190
xaa xaa xaa xaa xaa Glu 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

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<210> 156
<211> 59
<212> PRT
<213> Artificial sequence

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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>
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<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
<220>
<221> Variant
<222> (16)..(16)
<223> xaa in position 16 is Ile, Leu or Val
<220>
<221> Variant

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<222> (21)..(21)
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<222> (25)..(25)
<223> xaa in position 25 is Ala or Ser
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<222> (28)..(29)
<223> xaa in position 28 to 29 is any amino acid
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<222> (32)..(32)
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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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<222> (35)..(35)
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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>
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<222> (42)..(42)
<223> xaa in position 42 is Asp or Glu
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<222> (48)..(48)
<223> xaa in position 48 is Ala or Ile
<220>
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<222> (52)..(52)
<223> xaa in position 52 is any amino acid
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<222> (53)..(53)
<223> xaa in position 53 is Ile or Val
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<221> Variant
<222> (56)..(56)
<223> xaa in position 56 is any amino acid
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<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
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 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
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 Leu Asn Ser xaa xaa His Val xaa xaa Tyr xaa
 50 55

<210> 157
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 <213> Artificial sequence

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 <221> Variant
 <222> (5)..(5)
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 <220>
 <221> Variant
 <222> (6)..(6)
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 <221> Variant
 <222> (7)..(7)
 <223> xaa in position 7 is Gu or Gly
 <220>
 <221> Variant
 <222> (8)..(10)
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 <223> xaa in position 11 is Gln or Ser
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 <222> (13)..(16)
 <223> xaa in position 13 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> (20)..(20)
 <223> xaa in position 20 is Phe or Trp
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 <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

<210> 158
 <211> 16
 <212> PRT
 <213> Artificial sequence

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 <223> xaa in position 3 is any amino acid
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 <222> (6)..(8)
 <223> xaa in position 6 to 8 is any amino acid
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 <223> xaa in position 9 is any or no amino acid
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 <223> xaa in position 13 is Ala or Ser
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 <221> Variant
 <222> (14)..(14)
 <223> xaa in position 14 is any amino acid
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 <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
 G n xaa xaa G n Phe xaa xaa xaa xaa Val xaa G n 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
 Seite 199

<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

PF58307. txt

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cct gcaggt c aacat ggt gg agcacgacac act t gt ct ac t ccaaaaat a t caaagat ac 60
agt ct cagaa gaccaaaggg caat t gagac t t t t caacaa aggggt aat at ccggaaacct 120
cct cggat t c cat t gccag ct at ct gt ca ct t t at t gt g aagat agt gg aaaaggaagg 180
t ggct cct ac aaat gccat c at t gcgat aa aggaaaggcc at cgt t gaag at gcct ct gc 240
cgacagt ggt cccaaagat g gacccccacc cacgaggagc at cgt ggaaa aagaagacgt 300
t ccaaccacg t ct t caaagc aagt ggat t g at gt gat aac at ggt ggagc acgacacact 360
t gt ct act cc aaaaat at ca aagat acagt ct cagaagac caaagggcaa t t gagact t t 420
t caacaagg gt aat at ccg gaaacct cct cggat t ccat t gccagct a t ct gt cact t 480
t at t gt gaag at agt ggaaa aggaaggt gg ct cct acaaa t gccat cat t gcgat aaagg 540
aaaggccat c gt t gaagat g cct ct gccga cagt ggt ccc aaagat ggac ccccaccac 600
gaggagcat c gt ggaaaaag aagacgt t cc aaccacgt ct t caaagcaag t ggat t gat g 660
t gat at ct cc act gacgt aa gggat gacgc acaat cccac t at cct t cgc aagaccct t c 720
ct ct at at aa ggaagt t cat t t cat t t gga gagga 755

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<210> 162
 <211> 211
 <212> DNA
 <213> Cauliflower mosaic virus

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<400> 162
agt ccgcaa aat caccagt ct ct ct ct ac aat ct at ct ct ct ct at t t t ct ccagaa 60
t aat gt gt ga gt agt t ccca gat aagggaa t t aggggt t ct t at aggggt t t cgct cat gt g 120
t t gagcat at aagaaacct t agt at gt at t t gt at t t gt aaaat act t c t at caat aaa 180
at t t ct aat t cct aaaacca aaat ccagt g a 211

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<210> 163
 <211> 819
 <212> DNA
 <213> Thalassiosira pseudonana

<220>
 <221> CDS
 <222> (1)..(819)

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<400> 163
at g gac gcc t ac aac gct gca at g gat aag at c ggt gcc gcc at c at c 48
Met Asp Ala Tyr Asn Ala Ala Met Asp Lys Ile Gly Ala Ala Ile Ile
1 5 10 15
gat t gg t ct gat ccc gat gga aag t t c cgt gcc gat aga gag gac t gg 96
Asp Trp Ser Asp Pro Asp Gly Lys Phe Arg Ala Asp Arg Glu Asp Trp
20 25 30
t gg ct c t gc gac t t c cgt agc gcc at c acc at c gcc ct c at c t ac at c 144
Trp Leu Cys Asp Phe Arg Ser Ala Ile Thr Ile Ala Leu Ile Tyr Ile
35 40 45
gcc t t c gt c at c ct c ggt t cc gcc gt c at g caa t cc ct c ccc gca at g 192
Ala Phe Val Ile Leu Gly Ser Ala Val Met Gl n Ser Leu Pro Ala Met
50 55 60
gat ccc t ac ccc at c aaa t t c ct c t ac aac gt c t cc caa at c t t c ct t 240
Asp Pro Tyr Pro Ile Lys Phe Leu Tyr Asn Val Ser Gl n Ile Phe Leu
65 70 75 80
t gt gcc t ac at g act gt c gag gcg gga t t t t t g gcc t ac cgc aat gga 288
Cys Ala Tyr Met Thr Val Gu Ala Gly Phe Leu Ala Tyr Arg Asn Gly
85 90 95
t at acc gt c at g cct t gc aat cat t t c aat gt g aat gat cct ccc gt g 336
Tyr Thr Val Met Pro Cys Asn His Phe Asn Val Asn Asp Pro Pro Val
100 105 110
gcg aat ct t ct t t gg t t g t t t t at at t t cc aag gt g t gg gac t t t t gg 384
Ala Asn Leu Leu Trp Leu Phe Tyr Ile Ser Lys Val Trp Asp Phe Trp
115 120 125
gat acc att t t c att gt g t t g ggg aag aag t gg cgt caa t t a t ct t t c 432
Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gl n Leu Ser Phe
130 135 140
t t g cat gt a t ac cat cac acc acc at c t t t ct a t t c t at t gg ct g aat 480
Leu His Val Tyr His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
145 150 155 160
gcc aat gt c t t g t ac gat ggt gac at c t t c ct t acc at c t t g ct c aat 528
Ala Asn Val Leu Tyr Asp Gly Asp Ile Phe Leu Thr Ile Leu Leu Asn
165 170 175

```

PF58307.txt

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
acc aaa gat tcc aag acg ggc aag agt ctt cct ata tgg tgg aag tcc	624
Thr Lys Asp Ser Lys Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser	
	195
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
gct acc tac ctt gtc ttc cac ggg tgt gat aag gtg tcc cgt atc	720
Ala Thr Tyr Leu Val Phe His Gly Cys Asp Lys Val Ser Leu Arg Ile	
	225
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
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
t ag	819

<210> 164
 <211> 272
 <212> PRT
 <213> Thal assi osi r a pseudonana

<400> 164	
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 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 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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 <212> DNA
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<220>
 <221> CDS
 <222> (1).. (837)

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 ct t ggc gac t ct att ct g agc t gg gcc gac cct gat cac t at cg t gga 96
 Leu Gly Asp Ser Ile Leu Ser Tr p Ala Asp Pro Asp His Tyr Arg Gly
 20 25 30
 cat acc gag gga t gg gag t t c act gac t t t t ct gct gct t t t agc at t 144
 His Thr Gl u Gly Tr p Gl u Phe Thr Asp Phe Ser Ala Ala Phe Ser Ile
 35 40 45
 gcc gt c gcg t ac ct c ct g t t t gt c t t t gt t gga t ct ct c at t at g agt 192
 Ala Val Ala Tyr Leu Leu Phe Val Phe Val Gly Ser Leu Ile Met Ser
 50 55 60
 at g gga gt c ccc gca at t gac cct t at ccg ct c aag t t t gt c t ac aat 240
 Met Gly Val Pro Ala Ile Asp Pro Tyr Pro Leu Lys Phe Val Tyr Asn
 65 70 75 80
 gt t t ca cag att at g ct t t gt gct t ac at g acc at t gaa gcc agt ct t 288
 Val Ser Gl n Ile Met Leu Cys Ala Tyr Met Thr Ile Gl u Ala Ser Leu
 85 90 95
 ct a gct t at cg t aac ggc t ac aca t t c t gg cct t gc aac gat t gg gac 336
 Leu Ala Tyr Arg Asn Gly Tyr Thr Phe Tr p Pro Cys Asn Asp Tr p Asp
 100 105 110
 t t t gaa aag ccg cct at c gct aag ct c ct c t gg ct c t t t t ac gt t t cc 384
 Phe Gl u Lys Pro Pro Ile Ala Lys Leu Leu Tr p Leu Phe Tyr Val Ser
 115 120 125
 aaa att t gg gat t t t t gg gac acc at c t t t at t gt t ct c ggg aag aag 432
 Lys Ile Tr p Asp Phe Tr p Asp Thr Ile Phe Ile Val Leu Gly Lys Lys
 130 135 140
 t gg cg t caa ct t t cc t t c ct g cac gt c t ac cat cac acc acc at c t t t 480
 Tr p Arg Gl n Leu Ser Phe Leu His Val Tyr His His Thr Thr Ile Phe
 145 150 155 160
 ct c t t c t ac t gg t t g aat gca cat gt a aac t t t gat ggt gat at t t t c 528
 Leu Phe Tyr Tr p Leu Asn Ala His Val Asn Phe Asp Gly Asp Ile Phe
 165 170 175
 ct c acc at c gt c t t g aac ggt t t c at c cac acc gt c at g t ac acg t ac 576
 Leu Thr Ile Val Leu Asn Gly Phe Ile His Thr Val Met Tyr Thr Tyr
 180 185 190
 t ac t t c att t gc at g cac acc aag gt c cca gag acc ggc aaa t cc t t g 624
 Tyr Phe Ile Cys Met His Thr Lys Val Pro Gl u Thr Gly Lys Ser Leu
 195 200 205
 ccc att t gg t gg aaa t ct agt t t g aca agc at g cag ct g gt g cag t t c 672
 Pro Ile Tr p Tr p Lys Ser Ser Leu Thr Ser Met Gl n Leu Val Gl n Phe
 210 215 220
 at c acg at g at g acg cag gct at c at g at c t t g t ac aag ggc t gt gct 720
 Ile Thr Met Met Thr Gl n Ala Ile Met Ile Leu Tyr Lys Gly Cys Ala
 225 230 235 240
 gct ccc cat agc cgg gt g gt g aca t cg t ac t t g gt t t ac at t t t g t cg 768
 Ala Pro His Ser Arg Val Val Thr Ser Tyr Leu Val Tyr Ile Leu Ser
 245 250 255
 ct c t t t att t t g t t c gcc cag t t c t t t gt c agc t ca t ac ct c aag ccg 816
 Leu Phe Ile Leu Phe Ala Gl n Phe Phe Val Ser Ser Tyr Leu Lys Pro
 260 265 270
 aag aag aag aag aca gct t aa 837
 Lys Lys Lys Lys Thr Ala

<210> 166
 <211> 278
 <212> PRT
 <213> Phaeodactylum tricornutum

<400> 166
 Met Met Val Pro Ser Ser Tyr Asp Gl u 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
 His Thr Glu Gly Trp Gu Phe Thr Asp Phe Ser Ala Ala Phe Ser Ile
 Ala Val Ala Tyr Leu Leu Phe Val Phe Val Gly Ser Leu Ile Met Ser
 Met Gly Val Pro Ala Ile Asp Pro Tyr Pro Leu Lys Phe Val Tyr Asn
 Val Ser Gln Ile Met Leu Cys Ala Tyr Met Thr Ile Glu Ala Ser Leu
 Leu Ala Tyr Arg Asn Gly Tyr Thr Phe Trp Pro Cys Asn Asp Trp Asp
 Phe Gu Lys Pro Pro Ile Ala Lys Leu Leu Trp Leu Phe Tyr Val Ser
 Lys Ile Trp Asp Phe Trp Asp Thr Ile Phe Ile Val Leu Gly Lys Lys
 Trp Arg Gln Leu Ser Phe Leu His Val Tyr His His Thr Thr Ile Phe
 Leu Phe Tyr Trp Leu Asn Ala His Val Asn Phe Asp Gly Asp Ile Phe
 Leu Thr Ile Val Leu Asn Gly Phe Ile His Thr Val Met Tyr Thr Tyr
 Tyr Phe Ile Cys Met His Thr Lys Val Pro Gu Thr Gly Lys Ser Leu
 Pro Ile Trp Trp Lys Ser Ser Leu Thr Ser Met Gln Leu Val Gln Phe
 Ile Thr Met Met Thr Gln Ala Ile Met Ile Leu Tyr Lys Gly Cys Ala
 Ala Pro His Ser Arg Val Val Thr Ser Tyr Leu Val Tyr Ile Leu Ser
 Leu Phe Ile Leu Phe Ala Gln Phe Phe Val Ser Ser Tyr Leu Lys Pro
 Lys Lys Lys Lys Thr Ala
 20
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<210> 167
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 <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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 <223> xaa in position 18 is any amino acid
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 <222> (22)..(23)
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<223> xaa in position 24 is any or no amino acid
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<222> (37)..(38)
<223> xaa in position 37 to 38 is any amino acid
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<222> (174)..(174)
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<222> (212)..(213)
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<222> (216)..(216)
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<222> (223)..(223)
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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)
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 <222> (247)..(248)
 <223> xaa in position 247 to 248 is any amino acid
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 <222> (261)..(261)
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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
 Asp xaa Tyr xaa xaa xaa xaa xaa xaa xaa Gly xaa xaa Ile xaa xaa
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 Trp xaa Asp Pro Asp xaa xaa xaa Arg xaa xaa xaa Gu 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 G n Ile xaa Leu
 65 70 75 80
 Cys Ala Tyr Met Thr xaa Gu 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 G n 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 G n Leu xaa G n Phe xaa xaa Met Met xaa G n
 210 215 220
 Ala xaa 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
 G n 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

<220>
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 <222> (27)..(27)
 <223> xaa in position 27 is any amino acid
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 <222> (29)..(29)
 <223> xaa in position 29 is any amino acid
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 <221> Variant
 <222> (30)..(34)
 <223> xaa in position 30 to 34 is any or no amino acid
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 <221> Variant
 <222> (39)..(39)
 <223> xaa in position 39 is Leu or Val

 <400> 168
 G y Lys Lys Trp Arg G n Leu Ser Phe Leu H i s Val Tyr H i s H i s 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 G y Phe Ile H i s Thr Val Met
 35 40 45
 Tyr Thr Tyr Tyr Phe Ile Cys Met H i s Thr Lys
 50 55

<210> 169
 <211> 60
 <212> PRT
 <213> Artificial sequence

<220>
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 <223> xaa in position 7 is Phe or Met
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 <221> Variant
 <222> (14)..(14)
 <223> xaa in position 14 is Ile or Val
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 <222> (17)..(17)
 <223> xaa in position 17 is any amino acid
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 <222> (18)..(18)
 <223> xaa in position 18 is any or no amino acid
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 <222> (20)..(20)
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 <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
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 <221> Variant
 <222> (33)..(33)
 <223> xaa in position 33 is Asp or H i s
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 <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
 Met Asp Val Val Glu Gln Gln Trp Arg Arg Phe Val Asp Ala Val Asp
 Seite 210

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1          5          10          15
aac gga at c gt g gag t t c at g gag cat gag aag ccc aac aag ct g aac      96
Asn Gly Ile Val Gu Phe Met Gu His Gu Lys Pro Asn Lys Leu Asn
20
gag ggc aag ct c t t c acc t cg acc gag gag at g at g gcg ct t at c gt c      144
Gu Gy Lys Leu Phe Thr Ser Thr Gu Gu Met Met Ala Leu Ile Val
35
ggc t ac ct g gcg t t c gt g gt c ct c ggg t cc gcc t t c at g aag gcc t t t      192
Gy Tyr Leu Ala Phe Val Val Leu Gy Ser Ala Phe Met Lys Ala Phe
50
gt c gat aag cct t t c gag ct c aag t t c ct c aag ct c gt g cac aac at c      240
Val Asp Lys Pro Phe Gu Leu Lys Phe Leu Lys Leu Val His Asn Ile
65
t t c ct c acc ggt ct g t cc at g t ac at g gcc acc gag t gc gcg cgc cag      288
Phe Leu Thr Gy Leu Ser Met Tyr Met Ala Thr Gu Cys Ala Arg Gn
85
gca t ac ct c ggc ggc t ac aag ct c t t t ggc aac ccg at g gag aag ggc      336
Ala Tyr Leu Gy Gy Tyr Lys Leu Phe Gy Asn Pro Met Gu Lys Gy
100
acc gag t cg cac gcc ccg ggc at g gcc aac at c at c t ac at c t t c t ac      384
Thr Gu Ser His Ala Pro Gy Met Ala Asn Ile Ile Tyr Ile Phe Tyr
115
gt g agc aag t t c ct c gaa t t c ct c gac acc gt c t t c at g at c ct c ggc      432
Val Ser Lys Phe Leu Gu Phe Leu Asp Thr Val Phe Met Ile Leu Gy
130
aag aag t gg aag cag ct c agc t t t ct c cac gt c t ac cac cac gcg agc      480
Lys Lys Trp Lys Gn Leu Ser Phe Leu His Val Tyr His His Ala Ser
145
at c agc t t c at c t gg ggc at c at c gcc cgc t t c gcg ccc ggt ggc gac      528
Ile Ser Phe Ile Trp Gy Ile Ile Ala Arg Phe Ala Pro Gy Gy Asp
165
gcc t ac t t c t ct acc at c ct c aac agc agc gt g cat gt c gt g ct c t ac      576
Ala Tyr Phe Ser Thr Ile Leu Asn Ser Ser Val His Val Val Leu Tyr
180
ggc t ac t ac gcc t cg acc acc ct c ggc t ac acc t t c at g cgc ccg ct g      624
Gy Tyr Tyr Ala Ser Thr Thr Leu Gy Tyr Thr Phe Met Arg Pro Leu
195
cgc ccg t ac att acc acc att cag ct c acg cag t t c at g gcc at g gt c      672
Arg Pro Tyr Ile Thr Thr Ile Gn Leu Thr Gn Phe Met Ala Met Val
210
gt c cag t cc gt c t at gac t ac t ac aac ccc t gc gac t ac ccg cag ccc      720
Val Gn Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gn Pro
225
ct c gt c aag ct g ct c t t c t gg t ac at g ct c acc at g ct c ggc ct c t t c      768
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu Gy Leu Phe
245
ggc aac t t c t t c gt g cag cag t ac ct c aag ccc aag gcg ccc aag aag      816
Gy Asn Phe Phe Val Gn Gn Tyr Leu Lys Pro Lys Ala Pro Lys Lys
260
cag aag acc at c t aa
Gn Lys Thr Ile
275

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<210> 172
 <211> 276
 <212> PRT
 <213> Traust ochyt r i u m sp.

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<400> 172
Met Asp Val Val Gu Gn Gn Trp Arg Arg Phe Val Asp Ala Val Asp
1          5          10          15
Asn Gly Ile Val Gu Phe Met Gu His Gu Lys Pro Asn Lys Leu Asn
20
Gu Gy Lys Leu Phe Thr Ser Thr Gu Gu Met Met Ala Leu Ile Val
35
Gy Tyr Leu Ala Phe Val Val Leu Gy Ser Ala Phe Met Lys Ala Phe
50
Val Asp Lys Pro Phe Gu Leu Lys Phe Leu Lys Leu Val His Asn Ile
Sei te 211

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65 Phe Leu Thr Gly Leu Ser Met Tyr Met Ala Thr Gu Cys Ala Arg Gn
 70 75 80
 85 90 95
 Ala Tyr Leu Gly Tyr Lys Leu Phe Gly Asn Pro Met Gu Lys Gly
 100 105 110
 Thr Gu Ser His Ala Pro Gly Met Ala Asn Ile Ile Tyr Ile Phe Tyr
 115 120 125
 Val Ser Lys Phe Leu Gu Phe Leu Asp Thr Val Phe Met Ile Leu Gly
 130 135 140
 Lys Lys Trp Lys Gn 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 Gn Leu Thr Gn Phe Met Ala Met Val
 210 215 220
 Val Gn Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro Gn 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 Gn Gn Tyr Leu Lys Pro Lys Ala Pro Lys Lys
 260 265 270
 Gn Lys Thr Ile
 275

<210> 173
 <211> 1047
 <212> DNA
 <213> Marchantia polymorpha

<220>
 <221> CDS
 <222> (1)..(1047)

<400> 173
 atg gcg acg aag agc ggg agc gga ttg ctg gag tgg ata gca gta gcg 48
 Met Ala Thr Lys Ser Gly Ser Gly Leu Leu Gu 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 Gn Ala Arg Ser Ser Pro Gu Gy Gu Ile Val Gy
 20 25 30
 ggg aat agg atg ggc tct gga aac gga gct gag tgg acc acg agt ctg 144
 Gy Asn Arg Met Gy Ser Gy Asn Gy Ala Gu Trp 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 Gy Lys Ser Gy Gy Ala Ser
 50 55 60
 aaa gtg agg cct ctg gag gag aga atc ggg gag gcg gtg ttc aga gtt 240
 Lys Val Arg Pro Leu Gu Gu Arg Ile Gy Gu 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 Gu Asp Val Val Gy 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 Gu Ser Pro Val Pro Val Leu Ala Cys Ile
 100 105 110
 tct ctg tac ttg ctg 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 ctg gcc ctg cgt tgc ctt 432
 Ser Gy Gn Lys Pro Arg Lys Gu 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

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gt c	ggt	ct c	att	gcc	gca	gct	cga	cat	t ac	ggg	t at	agt	gt a	t gg	ggg	528
Val	G y	Leu	I l e	Al a	Al a	Al a	Arg	Hi s	Tyr	G y	Tyr	Ser	Val	Tr p	G y	
				165				170						175		
aac	t ac	t ac	aga	gaa	aga	gaa	ccc	gca	at g	aat	t t g	ct c	at t	t ac	gt g	576
Asn	Tyr	Tyr	Arg	G u	Arg	G u	Pro	Al a	Met	Asn	Leu	Leu	I l e	Tyr	Val	
			180					185					190			
t t c	t ac	at g	t cg	aag	ct g	t ac	gaa	t t t	at g	gac	acg	gcc	at t	at g	t t a	624
Phe	Tyr	Met	Ser	Lys	Leu	Tyr	G u	Phe	Met	Asp	Thr	Al a	I l e	Met	Leu	
			195				200					205				
t t c	aga	aga	aat	ct g	cga	caa	gt c	acg	t ac	t t g	cat	gt a	t at	cac	cac	672
Phe	Arg	Arg	Asn	Leu	Arg	G n	Val	Thr	Tyr	Leu	Hi s	Val	Tyr	Hi s	Hi s	
	210					215					220					
gca	agc	at c	gca	at g	at t	t gg	t gg	at a	at t	t gc	t at	cgg	t t t	cca	gga	720
Al a	Ser	I l e	Al a	Met	I l e	Tr p	Tr p	I l e	I l e	Cys	Tyr	Arg	Phe	Pro	G y	
	225				230					235					240	
gct	gat	t cg	t at	t t c	t cc	gca	gca	t t c	aat	t cc	t gt	at c	cat	gt a	gcg	768
Al a	Asp	Ser	Tyr	Phe	Ser	Al a	Al a	Phe	Asn	Ser	Cys	I l e	Hi s	Val	Al a	
				245					250					255		
at g	t ac	ct g	t at	t at	ct a	ct c	gcg	gca	acc	gt c	gcc	aga	gac	gaa	aag	816
Met	Tyr	Leu	Tyr	Tyr	Leu	Leu	Al a	Al a	Thr	Val	Al a	Arg	Asp	G u	Lys	
			260					265					270			
cgg	aga	cgc	aaa	t at	ct c	t t c	t gg	gga	aag	t at	ct g	acc	at c	at a	caa	864
Arg	Arg	Arg	Lys	Tyr	Leu	Phe	Tr p	G y	Lys	Tyr	Leu	Thr	I l e	I l e	G n	
			275				280					285				
at g	ct t	cag	t t t	t t g	t cc	t t c	at t	ggg	cag	gcg	at t	t at	gca	at g	t gg	912
Met	Leu	G n	Phe	Leu	Ser	Phe	I l e	G y	G n	Al a	I l e	Tyr	Al a	Met	Tr p	
	290					295					300					
aag	t t t	gaa	t ac	t at	ccc	aag	ggc	t t t	ggc	agg	at g	t t g	t t c	t t t	t ac	960
Lys	Phe	G u	Tyr	Tyr	Pro	Lys	G y	Phe	G y	Arg	Met	Leu	Phe	Phe	Tyr	
	305				310					315					320	
t ct	gt a	t ca	t t g	t t g	gca	t t t	t t c	ggc	aac	t t c	t t t	gt c	aaa	aag	t at	1008
Ser	Val	Ser	Leu	Leu	Al a	Phe	Phe	G y	Asn	Phe	Phe	Val	Lys	Lys	Tyr	
					325				330					335		
t cg	aac	gct	t ca	cag	cct	aag	aca	gt t	aaa	gt g	gag	t ga				1047
Ser	Asn	Al a	Ser	G n	Pro	Lys	Thr	Val	Lys	Val	G u					
			340					345								

<210> 174
 <211> 348
 <212> PRT
 <213> Marchantia polymorpha

<400> 174
 Met Al a Thr Lys Ser G y Ser G y Leu Leu G u Trp I l e Al a Val Al a
 1 5 10
 Al a Lys Met Lys G n Al a Arg Ser Ser Pro G u G y G u I l e Val G y
 20 25 30
 G y Asn Arg Met G y Ser G y Asn G y Al a G u Trp Thr Thr Ser Leu
 35 40 45
 I l e Hi s Al a Phe Leu Asn Al a Thr Asn G y Lys Ser G y G y Al a Ser
 50 55 60
 Lys Val Arg Pro Leu G u G u Arg I l e G y G u Al a Val Phe Arg Val
 65 70 75 80
 Leu G u Asp Val Val G y Val Asp I l e Arg Lys Pro Asn Pro Val Thr
 85 90 95
 Lys Asp Leu Pro Met Val G u Ser Pro Val Pro Val Leu Al a Cys I l e
 100 105 110
 Ser Leu Tyr Leu Leu Val Val Tr p Leu Tr p Ser Ser Hi s I l e Lys Al a
 115 120 125
 Ser G y G n Lys Pro Arg Lys G u Asp Pro Leu Al a Leu Arg Cys Leu
 130 135 140
 Val I l e Al a Hi s Asn Leu Phe Leu Cys Cys Leu Ser Leu Phe Met Cys
 145 150 155 160
 Val G y Leu I l e Al a Al a Arg Hi s Tyr G y Tyr Ser Val Tr p G y
 165 170 175
 Asn Tyr Tyr Arg G u Arg G u Pro Al a Met Asn Leu Leu I l e Tyr Val
 180 185 190
 Phe Tyr Met Ser Lys Leu Tyr G u Phe Met Asp Thr Al a I l e Met Leu

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	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								
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> Thr aust ochyt ri um sp

<220>
 <221> CDS
 <222> (1)..(831)

<400> 175																				
at g	gat	gt c	gt c	gag	cag	caa	t gg	cgc	cgc	t t c	gt g	gac	gcc	gt g	gac					48
Met	Asp	Val	Val	G u	G n	G n	Tr p	Arg	Arg	Phe	Val	Asp	Al a	Val	Asp					
1				5				10						15						
aac	gga	at c	gt g	gag	t t c	at g	gag	cat	gag	gag	ccc	aac	aag	ct g	aac					96
Asn	Gly	Ile	Val	G u	Phe	Met	G u	His	G u	G u	Pro	Asn	Lys	Leu	Asn					
			20				25					30								
gag	ggc	aag	ct c	t cc	acc	t cg	acc	gag	gag	at g	at g	gcg	ct t	at c	gt c					144
G u	G y	Lys	Leu	Ser	Thr	Ser	Thr	G u	G u	Met	Met	Al a	Leu	Ile	Val					
		35		40				45				45								
ggc	t ac	ct g	gcg	t t c	gt g	gt c	ct c	ggg	t cc	gcc	t t c	at g	aag	gcc	t t t					192
G y	Tyr	Leu	Al a	Phe	Val	Val	Leu	G y	Ser	Al a	Phe	Met	Lys	Al a	Phe					
	50			55				60				60								
gt c	gat	aag	cct	t t c	gag	ct c	aag	t t c	ct c	aag	ct c	gt g	cac	aac	at c					240
Val	Asp	Lys	Pro	Phe	G u	Leu	Lys	Phe	Leu	Lys	Leu	Val	His	Asn	Ile					
	65			70				75						80						
t t c	ct c	acc	ggg	ct g	t cc	at g	t ac	at g	gcc	acc	gag	t gc	gcg	cgc	cag					288
Phe	Leu	Thr	G y	Leu	Ser	Met	Tyr	Met	Al a	Thr	G u	Cys	Al a	Arg	G n					
			85					90				95								
gca	t ac	ct c	ggc	ggc	t ac	aag	ct c	t t t	ggc	aac	ccg	at g	gag	aag	ggc					336
Al a	Tyr	Leu	G y	G y	Tyr	Lys	Leu	Phe	G y	Asn	Pro	Met	G u	Lys	G y					
			100					105					110							
acc	gag	t cg	cac	gcc	ccg	ggc	at g	gcc	aac	at c	at c	t ac	at c	t t c	t ac					384
Thr	G u	Ser	His	Al a	Pro	G y	Met	Al a	Asn	Ile	Ile	Tyr	Ile	Phe	Tyr					
		115		120				125				125								
gt g	agc	aag	t t c	ct c	gaa	t t c	ct c	gac	acc	gt c	t t c	at g	at c	ct c	ggc					432
Val	Ser	Lys	Phe	Leu	G u	Phe	Leu	Asp	Thr	Val	Phe	Met	Ile	Leu	Gly					
		130				135		140				140								
aag	aag	t gg	aag	cag	ct c	agc	t t t	ct c	cac	gt c	t ac	cac	cac	gcg	agc					480
Lys	Lys	Trp	Lys	G n	Leu	Ser	Phe	Leu	His	Val	Tyr	His	His	Al a	Ser					
				150				155						160						
at c	agc	t t c	at c	t gg	ggc	at c	at c	gcc	cgc	t t c	gcg	ccc	ggg	ggc	gac					528
Ile	Ser	Phe	Ile	Trp	G y	Ile	Ile	Al a	Arg	Phe	Al a	Pro	G y	G y	Asp					
				165				170					175							
gcc	t ac	t t c	t ct	acc	at c	ct c	aac	agc	agc	gt g	cat	gt c	gt g	ct c	t ac					576
Al a	Tyr	Phe	Ser	Thr	Ile	Leu	Asn	Ser	Ser	Val	His	Val	Val	Leu	Tyr					
			180					185				190								
ggc	t ac	t ac	gcc	t cg	acc	acc	ct c	ggc	t ac	acc	t t c	at g	cgc	ccg	ct g					624
G y	Tyr	Tyr	Al a	Ser	Thr	Thr	Leu	G y	Tyr	Thr	Phe	Met	Arg	Pro	Leu					

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195          200          205
cgc ccg tac att acc acc att cag ct c acg cag t t c at g gcc at g gt c      672
Arg Pro Tyr lle Thr Thr lle G n Leu Thr G n Phe Met Ala Met Val
210
gt c cag t cc gt c t at gac t ac t ac aac ccc t gc gac t ac ccg cag ccc      720
Val G n Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro G n Pro
225          230          235          240
ct c gt c aag ct g ct c t t c t gg t ac at g ct c acc at g ct c ggc ct c t t c      768
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu G y Leu Phe
245          250          255
ggc aac t t c t t c gt g cag cag t ac ct c aag ccc aag gcg ccc aag aag      816
G y Asn Phe Phe Val G n G n Tyr Leu Lys Pro Lys Ala Pro Lys Lys
260          265          270
cag aag acc at c t aa
G n Lys Thr lle
275

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<210> 176
 <211> 276
 <212> PRT
 <213> Thr aust ochyt ri um sp

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<400> 176
Met Asp Val Val G u G n G n Trp Arg Arg Phe Val Asp Ala Val Asp
1          5          10
Asn G y lle Val G u Phe Met G u His G u G u Pro Asn Lys Leu Asn
20          25          30
G u G y Lys Leu Ser Thr Ser Thr G u G u Met Met Ala Leu lle Val
35          40          45
G y Tyr Leu Ala Phe Val Val Leu G y Ser Ala Phe Met Lys Ala Phe
50          55          60
Val Asp Lys Pro Phe G u Leu Lys Phe Leu Lys Leu Val His Asn lle
65          70          75          80
Phe Leu Thr G y Leu Ser Met Tyr Met Ala Thr G u Cys Ala Arg G n
85          90          95
Ala Tyr Leu G y G y Tyr Lys Leu Phe G y Asn Pro Met G u Lys G y
100          105          110
Thr G u Ser His Ala Pro G y Met Ala Asn lle lle Tyr lle Phe Tyr
115          120          125
Val Ser Lys Phe Leu G u Phe Leu Asp Thr Val Phe Met lle Leu G y
130          135          140
Lys Lys Trp Lys G n Leu Ser Phe Leu His Val Tyr His His Ala Ser
145          150          155          160
lle Ser Phe lle Trp G y lle lle Ala Arg Phe Ala Pro G y G y Asp
165          170          175
Ala Tyr Phe Ser Thr lle Leu Asn Ser Ser Val His Val Val Leu Tyr
180          185          190
G y Tyr Tyr Ala Ser Thr Thr Leu G y Tyr Thr Phe Met Arg Pro Leu
195          200          205
Arg Pro Tyr lle Thr Thr lle G n Leu Thr G n Phe Met Ala Met Val
210          215          220
Val G n Ser Val Tyr Asp Tyr Tyr Asn Pro Cys Asp Tyr Pro G n Pro
225          230          235          240
Leu Val Lys Leu Leu Phe Trp Tyr Met Leu Thr Met Leu G y Leu Phe
245          250          255
G y Asn Phe Phe Val G n G n Tyr Leu Lys Pro Lys Ala Pro Lys Lys
260          265          270
G n Lys Thr lle
275

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<210> 177
 <211> 879
 <212> DNA
 <213> Ostreococcus tauri

<220>
 <221> CDS
 <222> (1).. (879)

<400> 177
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 Thr Ala His Leu Pro Ala
35 40 45
att gaa tcc cct acc cca ctg gtg act agc ct c 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
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 ct c 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 ct c 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 ct c 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 ct a 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 cca 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 ct a 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 ct a acg caa atg cag atg ctt cag ttt ttc ttc 720
Trp Trp Gly Arg His Leu Thr Gln Met Gln 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 ct c gtc tat atg atg agc ctt ct c 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 ct c cag 864
Phe Gly His Phe Tyr Tyr Ser Lys His Ile 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
Met Ser Gly Leu Arg Ala Pro Asn Phe Leu His Arg Phe Trp Thr Lys
1 5 10 15

PF58307. txt

Trp Asp Tyr Ala Ile Ser Lys Val Val Phe Thr Cys Ala Asp Ser Phe
 20 25 30
 Gn Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
 35 40 45
 Ile Gu 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
 Ile Arg Gu 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 Gn
 100 105 110
 Ala Tyr Gn Asn Gly Tyr Thr Leu Trp Gly Asn Gu Phe Lys Ala Thr
 115 120 125
 Gu Thr Gn Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
 130 135 140
 Tyr Gu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
 145 150 155
 Gn 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 Gu Asp Pro Lys Arg Ser Asn Tyr Leu
 210 215 220
 Trp Trp Gly Arg His Leu Thr Gn Met Gn Met Leu Gn Phe Phe Phe
 225 230 235
 Asn Val Leu Gn 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 Gn
 275 280 285
 Lys Lys Gn Gn
 290

<210> 179
 <211> 873
 <212> DNA
 <213> Marchantia polymorpha

<220>
 <221> CDS
 <222> (1)..(873)

<400> 179
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 Met Gu Ala Tyr Gu 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
 Gu Thr Leu Gn Arg Leu Arg Gly Gly Val Val Leu Thr Gu 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 Ile Val Leu
 35 40 45
 ggg ttg tcg tcc tac ttg aca ttc gtg ttt ctg ggg ctg 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 Gu Pro Ala Ile Leu Asn
 65 70 75 80
 ctg ttt gtg atc ttc cac aac ttc gtc tgc ttc gca ctg 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 ctg aac agg tac tct ctg 336
 Met Cys Val Gly Ile Val Arg Gn Ala Ile Leu Asn Arg Tyr Ser Leu
 100 105 110

PF58307. txt

t gg ggc aat gcg t ac aat ccc aaa gaa gt t caa at g ggc cac ct g ct c 384
 Trp Gly Asn Ala Tyr Asn Pro Lys Gu Val Gn Met Gly His Leu Leu
 115 120 125
 t ac att ttc t ac at g t ca aag t ac at c gag ttt at g gac acg gt c at t 432
 Tyr lle Phe Tyr Met Ser Lys Tyr lle Gu Phe Met Asp Thr Val lle
 130 135 140
 at g att ttg aag cgc aac acg cgc cag at c act gt g tt g cat gt g t ac 480
 Met lle Leu Lys Arg Asn Thr Arg Gn lle Thr Val Leu His Val Tyr
 145 150 155
 cac cac gca tcc at c tcc ttc at c tgg tgg at c at c gcc t ac cat gct 528
 His His Ala Ser lle Ser Phe lle Trp Trp lle lle Ala Tyr His Ala
 165 170 175
 cct ggc ggt gaa gct t at ttc tct gcc gca ttg aac tcc gga gt a cat 576
 Pro Gly Gly Gu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
 180 185 190
 gt g ct c at g t ac ct c t ac t ac ct t ttg gca gca act ct g 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 t ac ct a tgg tgg gga aaa t ac ttg aca cag 672
 Gu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr Gn
 210 215 220
 ct g cag at g ttc cag ttt gt c ct t aac at g at t cag gct t ac t ac gat 720
 Leu Gn Met Phe Gn Phe Val Leu Asn Met lle Gn Ala Tyr Tyr Asp
 225 230 235 240
 at t aag aac aac t cg cct t ac cca caa ttt ttg at c cag at t ttg ttc 768
 lle Lys Asn Asn Ser Pro Tyr Pro Gn Phe Leu lle Gn lle Leu Phe
 245 250 255
 t ac t ac at g at c t cg ct t tta gcg ct a ttt gga aac ttt t ac gt t cac 816
 Tyr Tyr Met lle Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val His
 260 265 270
 aaa t ac gt a t ca gcg ccc gca aaa cct gcg aag at c aag agc aaa aag 864
 Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys lle Lys Ser Lys Lys
 275 280 285
 gca gaa t aa 873
 Ala Gu
 290

<210> 180
 <211> 290
 <212> PRT
 <213> Marchantia polymorpha

<400> 180
 Met Gu Ala Tyr Gu Met Val Asp Ser Phe Val Ser Lys Thr Val Phe
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 Gu Thr Leu Gn Arg Leu Arg Gly Gly Val Val Leu Thr Gu Ser Ala
 20 25 30
 lle Thr Lys Gly Leu Pro Cys Val Asp Ser Pro Thr Pro lle Val Leu
 35 40 45
 Gly Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gly Leu lle Val lle
 50 55 60
 Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Gu Pro Ala lle Leu Asn
 65 70 75 80
 Leu Phe Val lle Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr
 85 90 95
 Met Cys Val Gly lle Val Arg Gn Ala lle Leu Asn Arg Tyr Ser Leu
 100 105 110
 Trp Gly Asn Ala Tyr Asn Pro Lys Gu Val Gn Met Gly His Leu Leu
 115 120 125
 Tyr lle Phe Tyr Met Ser Lys Tyr lle Gu Phe Met Asp Thr Val lle
 130 135 140
 Met lle Leu Lys Arg Asn Thr Arg Gn lle Thr Val Leu His Val Tyr
 145 150 155 160
 His His Ala Ser lle Ser Phe lle Trp Trp lle lle Ala Tyr His Ala
 165 170 175
 Pro Gly Gly Gu 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

Seite 218

PF58307. txt

195 200 205
 G u Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr G n
 210 215 220
 Leu G n Met Phe G n Phe Val Leu Asn Met Ile G n Ala Tyr Tyr Asp
 225 230 235 240
 Ile Lys Asn Asn Ser Pro Tyr Pro G n Phe Leu Ile G n Ile Leu Phe
 245 250 255
 Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val Hi s
 260 265 270
 Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
 275 280 285
 Ala G u
 290

<210> 181
 <211> 873
 <212> DNA
 <213> Physcomitrella patens

<220>
 <221> CDS
 <222> (1).. (873)

<400> 181
 at g gag gt c gt g gag aga t t c t ac ggt gag t t g gat ggg aag gt c t c g 48
 Met G u Val Val G u Arg Phe Tyr G y G u Leu Asp G y Lys Val Ser
 1 5 10
 cag ggc gt g aat gca t t g ct g ggt agt t t t ggg gt g gag t t g acg gat 96
 G n G y Val Asn Ala Leu Leu G y Ser Phe G y Val G u Leu Thr Asp
 20 25 30
 acg ccc act acc aaa ggc t t g ccc ct c gt t gac agt ccc aca ccc at c 144
 Thr Pro Thr Lys G y Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
 35 40 45
 gt c ct c ggt gt t t ct gt a t ac t t g act at t gt c at t gga ggg ct t t t g 192
 Val Leu G y Val Ser Val Tyr Leu Thr Ile Val Ile G y G y Leu Leu
 50 55 60
 t gg at a aag gcc agg gat ct g aaa ccg cgc gcc t c g gag cca t t t t t g 240
 Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser G u Pro Phe Leu
 65 70 75 80
 ct c caa gct t t g gt g ct t gt g cac aac ct g t t c t gt t t t gcg ct c agt 288
 Leu G n Ala Leu Val Leu Val Hi s Asn Leu Phe Cys Phe Ala Leu Ser
 85 90 95
 ct g t at at g t gc gt g ggc at c gct t at cag gct at t acc t gg cgg t ac 336
 Leu Tyr Met Cys Val G y Ile Ala Tyr G n Ala Ile Thr Trp Arg Tyr
 100 105 110
 t ct ct c t gg ggc aat gca t ac aat cct aaa cat aaa gag at g gcg at t 384
 Ser Leu Trp G y Asn Ala Tyr Asn Pro Lys Hi s Lys G u Met Ala Ile
 115 120 125
 ct g gt a t ac t t g t t c t ac at g t ct aag t ac gt g gaa t t c at g gat acc 432
 Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val G u Phe Met Asp Thr
 130 135 140
 gt t at c at g at a ct g aag cgc agc acc agg caa at a agc t t c ct c cac 480
 Val Ile Met Ile Leu Lys Arg Ser Thr Arg G n Ile Ser Phe Leu Hi s
 145 150 155 160
 gt t t at cat cat t ct t ca at t t cc ct c at t t gg t gg gct at t gct cat 528
 Val Tyr Hi s Hi s Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala Hi s
 165 170 175
 cac gct cct ggc ggt gaa gca t at t gg t ct gcg gct ct g aac t ca gga 576
 Hi s Ala Pro G y G y G u Ala Tyr Trp Ser Ala Ala Leu Asn Ser G y
 180 185 190
 gt g cat gt t ct c at g t at gcg t at t ac t t c t t g gct gcc t gc ct t cga 624
 Val Hi s Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
 195 200 205
 agt agc cca aag t ta aaa aat aag t ac ct t t t t gg ggc agg t ac t t g 672
 Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp G y Arg Tyr Leu
 210 215 220
 aca caa t t c caa at g t t c cag t t t at g ct g aac t ta gt g cag gct t ac 720
 Thr G n Phe G n Met Phe G n Phe Met Leu Asn Leu Val G n Ala Tyr

PF58307.txt

225 230 235 240
 t ac gac at g aaa acg aat gcg cca t at cca caa t gg ct g at c aag att 768
 Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gl n Trp Leu Ile Lys Ile
 245 250 255
 t t g t t c t ac t ac at g at c t cg t t g ct g t t t c t t ggc aat t t t t ac 816
 Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gl y Asn Phe Tyr
 260 265 270
 gt a caa aaa t ac at c aaa ccc t ct gac gga aag caa aag gga gct aaa 864
 Val Gl n Lys Tyr Ile Lys Pro Ser Asp Gl y Lys Gl n Lys Gl y Ala Lys
 275 280 285
 act gag t ga 873
 Thr Gl u
 290

<210> 182

<211> 290

<212> PRT

<213> Physcomitrella patens

<400> 182

Met Gl u Val Val Gl u Arg Phe Tyr Gl y Gl u Leu Asp Gl y Lys Val Ser
 1 5 10
 Gl n Gl y Val Asn Ala Leu Leu Gl y Ser Phe Gl y Val Gl u Leu Thr Asp
 20 25 30
 Thr Pro Thr Thr Lys Gl y Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
 35 40 45
 Val Leu Gl y Val Ser Val Tyr Leu Thr Ile Val Ile Gl y Gl y Leu Leu
 50 55 60
 Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Gl u Pro Phe Leu
 65 70 75 80
 Leu Gl n Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
 85 90 95
 Leu Tyr Met Cys Val Gl y Ile Ala Tyr Gl n Ala Ile Thr Trp Arg Tyr
 100 105 110
 Ser Leu Trp Gl y Asn Ala Tyr Asn Pro Lys His Lys Gl u Met Ala Ile
 115 120 125
 Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Gl u Phe Met Asp Thr
 130 135 140
 Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gl n 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 Gl y Gl y Gl u Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gl y
 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 Gl y Arg Tyr Leu
 210 215 220
 Thr Gl n Phe Gl n Met Phe Gl n Phe Met Leu Asn Leu Val Gl n Ala Tyr
 225 230 235 240
 Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gl n Trp Leu Ile Lys Ile
 245 250 255
 Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gl y Asn Phe Tyr
 260 265 270
 Val Gl n Lys Tyr Ile Lys Pro Ser Asp Gl y Lys Gl n Lys Gl y Ala Lys
 275 280 285
 Thr Gl u
 290

<210> 183

<211> 957

<212> DNA

<213> Mbrtierella alpina

<220>

<221> CDS

<222> (1)..(957)

PF58307. txt

<400> 183

at g	gag	t cg	att	gcg	cca	t t c	ct c	cca	t ca	aag	at g	ccg	caa	gat	ct g	48
Met	Gl u	Ser	I l e	Al a	Pro	Phe	Leu	Pro	Ser	Lys	Met	Pro	Gl n	Asp	Leu	
1				5					10					15		
t t t	at g	gac	ct t	gcc	acc	gct	at c	ggt	gt c	cgg	gcc	gcg	ccc	t at	gt c	96
Phe	Met	Asp	Leu	Al a	Thr	Al a	I l e	Gl y	Val	Arg	Al a	Al a	Pro	Tyr	Val	
			20					25					30			
gat	cct	ct c	gag	gcc	gcg	ct g	gt g	gcc	cag	gcc	gag	aag	t ac	at c	ccc	144
Asp	Pro	Leu	Gl u	Al a	Al a	Leu	Val	Al a	Gl n	Al a	Gl u	Lys	Tyr	I l e	Pro	
			35				40					45				
acg	att	gt c	cat	cac	acg	cgt	ggg	t t c	ct g	gt c	gcg	gt g	gag	t cg	cct	192
Thr	I l e	Val	Hi s	Hi s	Thr	Arg	Gl y	Phe	Leu	Val	Al a	Val	Gl u	Ser	Pro	
			50			55					60					
t t g	gcc	cgt	gag	ct g	ccg	t t g	at g	aac	ccg	t t c	cac	gt g	ct g	t t g	at c	240
Leu	Al a	Arg	Gl u	Leu	Pro	Leu	Met	Asn	Pro	Phe	Hi s	Val	Leu	Leu	I l e	
65					70				75						80	
gt g	ct c	gct	t at	t t g	gt c	acg	gt c	t t t	gt g	ggc	at g	cag	at c	at g	aag	288
Val	Leu	Al a	Tyr	Leu	Val	Thr	Val	Phe	Val	Gl y	Met	Gl n	I l e	Met	Lys	
				85					90					95		
aac	t t t	gag	cgg	t t c	gag	gt c	aag	acg	t t t	t cg	ct c	ct g	cac	aac	t t t	336
Asn	Phe	Gl u	Arg	Phe	Gl u	Val	Lys	Thr	Phe	Ser	Leu	Leu	Hi s	Asn	Phe	
			100					105					110			
t gt	ct g	gt c	t cg	at c	agc	gcc	t ac	at g	t gc	ggt	ggg	at c	ct g	t ac	gag	384
Cys	Leu	Val	Ser	I l e	Ser	Al a	Tyr	Met	Cys	Gl y	Gl y	I l e	Leu	Tyr	Gl u	
			115				120					125				
gct	t at	cag	gcc	aac	t at	gga	ct g	t t t	gag	aac	gct	gct	gat	cat	acc	432
Al a	Tyr	Gl n	Al a	Asn	Tyr	Gl y	Leu	Phe	Gl u	Asn	Al a	Al a	Asp	Hi s	Thr	
			130			135					140					
t t c	aag	ggt	ct t	cct	at g	gcc	aag	at g	at c	t gg	ct c	t t c	t ac	t t c	t cc	480
Phe	Lys	Gl y	Leu	Pro	Met	Al a	Lys	Met	I l e	Trp	Leu	Phe	Tyr	Phe	Ser	
145					150				155						160	
aag	at c	at g	gag	t t t	gt c	gac	acc	at g	at c	at g	gt c	ct c	aag	aag	aac	528
Lys	I l e	Met	Gl u	Phe	Val	Asp	Thr	Met	I l e	Met	Val	Leu	Lys	Lys	Asn	
				165					170					175		
aac	cgc	cag	at c	t cc	t t c	t t g	cac	gt t	t ac	cac	cac	agc	t cc	at c	t t c	576
Asn	Arg	Gl n	I l e	Ser	Phe	Leu	Hi s	Val	Tyr	Hi s	Hi s	Ser	Ser	I l e	Phe	
				180				185					190			
acc	at c	t gg	t gg	t t g	gt c	acc	t t t	gt t	gca	ccc	aac	ggt	gaa	gcc	t ac	624
Thr	I l e	Trp	Trp	Leu	Val	Thr	Phe	Val	Al a	Pro	Asn	Gl y	Gl u	Al a	Tyr	
				195			200					205				
t t c	t ct	gct	gcg	t t g	aac	t cg	t t c	at c	cat	gt g	at c	at g	t ac	ggc	t ac	672
Phe	Ser	Al a	Al a	Leu	Asn	Ser	Phe	I l e	Hi s	Val	I l e	Met	Tyr	Gl y	Tyr	
						215					220					
t ac	t t c	t t g	t cg	gcc	t t g	ggc	t t c	aag	cag	gt g	t cg	t t c	at c	aag	t t c	720
Tyr	Phe	Leu	Ser	Al a	Leu	Gl y	Phe	Lys	Gl n	Val	Ser	Phe	I l e	Lys	Phe	
225					230				235						240	
t ac	at c	acg	cgc	t cg	cag	at g	aca	cag	t t c	t gc	at g	at g	t cg	gt c	cag	768
Tyr	I l e	Thr	Arg	Ser	Gl n	Met	Thr	Gl n	Phe	Cys	Met	Met	Ser	Val	Gl n	
				245				250					255			
t ct	t cc	t gg	gac	at g	t ac	gcc	at g	aag	gt c	ct t	ggc	cgc	ccc	gga	t ac	816
Ser	Ser	Trp	Asp	Met	Tyr	Al a	Met	Lys	Val	Leu	Gl y	Arg	Pro	Gl y	Tyr	
			260					265					270			
ccc	t t c	t t c	at c	acg	gct	ct g	ct t	t gg	t t c	t ac	at g	t gg	acc	at g	ct c	864
Pro	Phe	Phe	I l e	Thr	Al a	Leu	Leu	Trp	Phe	Tyr	Met	Trp	Thr	Met	Leu	
				275			280					285				
ggt	ct c	t t c	t ac	aac	t t t	t ac	aga	aag	aac	gcc	aag	t t g	gcc	aag	cag	912
Gl y	Leu	Phe	Tyr	Asn	Phe	Tyr	Arg	Lys	Asn	Al a	Lys	Leu	Al a	Lys	Gl n	
					295				300							
gcc	aag	gcc	gac	gct	gcc	aag	gag	aag	gca	agg	aag	t t g	cag	t aa		957
Al a	Lys	Al a	Asp	Al a	Al a	Lys	Gl u	Lys	Al a	Arg	Lys	Leu	Gl n			
305					310				315							

<210> 184

<211> 318

<212> PRT

<213> Mbr t i e r e l l a a l p i n a

<400> 184

PF58307.txt

Met 1 Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
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> 185

<211> 251

<212> PRT

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<222> (17)..(23)

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<222> (117).. (119)
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<221> Vari ant
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<223> xaa in position 133 to 134 is any ami no aci d
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<222> (136).. (138)
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<222> (187).. (188)
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 <223> xaa in position 203 to 208 is any amino acid
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 <222> (209)..(209)
 <223> xaa in position 209 is any or no amino acid
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 <223> xaa in position 212 to 217 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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 <223> xaa in position 223 to 225 is any amino acid
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 <221> Variant
 <222> (227)..(227)
 <223> xaa in position 227 is any amino acid
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 <221> Variant
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 <223> xaa in position 233 to 246 is any amino acid
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 <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
 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 Gu Phe xaa Asp Thr xaa xaa Met xaa Leu xaa xaa
 100 105 110
 xaa xaa xaa Gn 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 Thr xaa xaa Gn xaa xaa Gn
 180 185 190
 Phe xaa xaa xaa xaa xaa Gn 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
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- <220>
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- <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
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- <223> xaa in position 19 is Lys or Arg
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- <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
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- <223> xaa in position 30 is Ala or Ser
- <220>
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- <223> xaa in position 31 is Ser or Thr
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- <222> (33)..(34)
- <223> xaa in position 33 to 34 is any amino acid

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 <222> (40)..(40)
 <223> xaa in position 40 is Ala or Thr
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 <222> (41)..(42)
 <223> xaa in position 41 to 42 is any amino acid
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 <223> xaa in position 45 is Gly or Asn
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 <222> (47)..(47)
 <223> xaa in position 47 is Asp or Glu
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 <222> (50)..(50)
 <223> xaa in position 50 is Phe or Trp
 <220>
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 <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
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 <222> (57)..(57)
 <223> xaa in position 57 is any amino acid
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 <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 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
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<223> xaa in position 3 is Leu or Met
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<220>
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<223> xaa in position 6 is Ala or Cys
<220>
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<222> (7)..(7)
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<220>
<221> Variant
<222> (8)..(8)
<223> xaa in position 8 is Glu or Gly
<220>
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<222> (9)..(9)
<223> xaa in position 9 is any amino acid
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<222> (10)..(10)
<223> xaa in position 10 is Ala, Ile or Val
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<222> (11)..(11)
<223> xaa in position 11 is any amino acid
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<222> (12)..(12)
<223> xaa in position 12 is Ala or Gln
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<223> xaa in position 19 is any amino acid
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<220>
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<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

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<212> PRT
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 <220>
 <221> Variant
 <222> (6)..(7)
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 <222> (8)..(8)
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 <221> Variant
 <222> (10)..(11)
 <223> xaa in position 10 to 11 is any amino acid
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 <222> (14)..(14)
 <223> xaa in position 14 is any amino acid
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 <222> (15)..(15)
 <223> xaa in position 15 is Ser or Thr
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 <222> (16)..(16)
 <223> xaa in position 16 is Leu or Met
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 <222> (21)..(22)
 <223> xaa in position 21 to 22 is any amino acid
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 <222> (24)..(24)
 <223> xaa in position 24 is Phe or Tyr

 <400> 188
 Tyr Pro 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

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 <211> 20
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 <222> (7)..(7)
 <223> xaa in position 7 is Leu or Met
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 <221> Variant
 <222> (8)..(8)
 <223> xaa in position 8 is any amino acid
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<221> Variant
 <222> (11)..(14)
 <223> xaa in position 11 to 14 is any amino acid
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 <222> (15)..(15)
 <223> xaa in position 15 is Gly, Ile or Val
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 <222> (17)..(17)
 <223> xaa in position 17 is Ala or Ser
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 <222> (18)..(18)
 <223> xaa in position 18 is any amino acid
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 <222> (19)..(19)
 <223> xaa in position 19 is Trp or Tyr
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 <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
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 <222> (13)..(13)
 <223> xaa in position 13 is Phe or Leu
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 <222> (15)..(15)
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 <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

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<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

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<221> Variant

<222> (7)..(8)

<223> xaa in position 7 to 8 is any amino acid

<220>

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<222> (9)..(9)

<223> xaa in position 9 is Ile, Leu or Val

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<222> (10)..(11)

<223> xaa in position 10 to 11 is any amino acid

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<222> (14)..(15)

<223> xaa in position 14 to 15 is any amino acid

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<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

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 <221> Variant
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 <222> (6)..(6)
 <223> xaa in position 6 is Trp or Tyr
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 <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> Phyt opt hor a i n f e s t a n s

<220>
 <221> CDS
 <222> (1)..(1086)

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 Met Ala Thr Lys Gu Ala Tyr Val Phe Pro Thr Leu Thr Gu Ile Lys
 1 5 10 15
 c g g t c g c t a c c t a a a g a c t g t t t c g a g g c t c g g t g c c t c t g t c g c t c 96
 Arg Ser Leu Pro Lys Asp Cys Phe Gu Ala Ser Val Pro Leu Ser Leu
 20 25 30
 t a c t a c a c c g t g c g t t g t c t g g t g a t c g c g g t g g c t a a c c t t c g g t 144
 Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ala Leu Thr Phe Gly
 35 40 45
 c t c a a c t a c g c t c g c g c t c t g c c c g a g g t c g a g a g c t t c t g g g c t c t g 192
 Leu Asn Tyr Ala Arg Ala Leu Pro Gu Val Gu Ser Phe Trp Ala Leu
 50 55 60
 g a c g c c g c a c t c t g c a c g g g c t a c a t c t t g c t g c a g g g c a t c g t g t t c 240
 Asp Ala Ala Leu Cys Thr Gy Tyr Ile Leu Leu Gn Gy Ile Val Phe
 65 70 75 80
 t g g g g c t t c t t c a c g g t g g g c c a c g a t g c c g g c c a c g g c g c c t t c t c g 288
 Trp Gy Phe Phe Thr Val Gy His Asp Ala Gy His Gy Ala Phe Ser
 85 90 95
 c g c t a c c a c c t g c t t a a c t t c g t g g t g g g c a c t t t c a t g c a c t c g c t c 336
 Arg Tyr His Leu Leu Asn Phe Val Val Gy Thr Phe Met His Ser Leu
 100 105 110
 a t c c t c a c g c c c t t c g a g t c g t g g a a g c t c a c g c a c c g t c a c c a c c a c 384
 Ile Leu Thr Pro Phe Gu 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 Gn Arg
130
aag gcc gac gac cac ccg ctg tct cgc aac ctg att ctg gcg ct c ggg      480
Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala Leu Gly
145
gca gcg tgg ct c 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
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 Gn Val Ser Ala
180
gtg gta atc tct ctt ct c gcc cac ttc gtg gcc gga ct c tcc at c      624
Val Val Ile Ser Leu Leu Ala His Phe Phe Val Ala Gly Leu Ser Ile
195
tat ctg agc ct c cag ctg gcc ctt aag acg atg gca at c tac tac tat      672
Tyr Leu Ser Leu Gn Leu Gly Leu Lys Thr Met Ala Ile Tyr Tyr Tyr
210
gga cct gtt ttt gtg ttc gcc agc atg ctg gtc att acc acc ttc ct a      720
Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu
225
cac cac aat gat gag gag acc cca tgg tac gcc gac t cg gag tgg acg      768
His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu Trp Thr
245
tac gtc aag gcc aac ct c t cg tcc gtg gac cga t cg tac gcc gcg ct c      816
Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala Leu
260
att gac aac ctg agc cac aac at c gcc acg cac cag at c cac cac ct t      864
Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gn Ile His His Leu
275
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Phe Pro Ile Ile Pro His Tyr Lys Leu Lys Lys Ala Thr Ala Ala Phe
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His Gn Ala Phe Pro Glu Leu Val Arg Lys Ser Asp Glu Pro Ile Ile
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aag gct ttc ttc cgg gt t gga cgt ct c tac gca aac tac gcc gt t gt g      1008
Lys Ala Phe Phe Arg Val Gly Arg Leu Tyr Ala Asn Tyr Gly Val Val
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Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His His His
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PF58307. txt

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 210 215 220
 Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu
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 His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu Trp Thr
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 Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gln Ile His His Leu
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 Ser Arg Tyr His Ser Val Asn Phe Ile Ile Gly Cys Ile Met His Ser
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 Ala Ile Leu Thr Pro Phe Glu Ser Trp Arg Val Thr His Arg His His
 115 120 125
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 His Lys Asn Thr Gly Asn Ile Asp Lys Asp Glu Ile Phe Tyr Pro His

PF58307.txt

Arg Ser Val Lys Asp Leu G n Asp Val Arg G n Trp Val Tyr Thr Leu
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 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
 Seite 240

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Phe Thr Val G y H i s Asp xaa G y H i s xaa Al a Phe Ser Arg Tyr H i s
85        90        95
xaa xaa Asn Phe xaa xaa G y xaa xaa M et H i s Ser xaa Ile Leu Thr
100       105       110
Pro Phe G u Ser Tr p xaa xaa Thr H i s Arg H i s H i s H i s Lys Asn Thr
115       120       125
G y Asn Ile Asp xaa Asp G u xaa Phe Tyr Pro xaa Arg xaa xaa xaa
130       135       140
Asp xaa xaa xaa xaa Arg xaa xaa xaa xaa xaa Leu G y xaa Al a Tr p
145       150       155       160
xaa xaa Tyr Leu xaa xaa G y xaa xaa Pro Arg xaa xaa xaa H i s Phe
165       170       175
xaa Pro xaa xaa Pro Leu xaa xaa Arg xaa xaa Ser Al a Val xaa xaa
180       185       190
Ser Leu xaa xaa xaa Al a xaa Phe Phe xaa Al a xaa xaa xaa Tyr
195       200       205
Leu xaa xaa xaa Leu G y xaa xaa xaa M et xaa xaa Tyr Tyr Tyr xaa
210       215       220
Pro xaa Phe Val Phe xaa Ser xaa Leu Val Ile Thr Thr Phe Leu H i s
225       230       235       240
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 20  25  30
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xaa His His Leu Phe Pro Ile Ile Pro His Tyr Lys
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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				
	50					55					60				



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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



Title:METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS

Publication No.US-2016-0369290-A1
Publication Date:12/22/2016

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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				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	
Sheet	1	of	4	Attorney Docket Number	074017-0013-01-US	

U. S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)				
	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		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
		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)					
	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		

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				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	
Sheet	2	of	4	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 <i>Arabidopsis</i> to Produce Nutritionally Important DHA in Seed Oil", <i>Functional Plant Biology</i> , 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.	
	CC	WOLFF, R. L., et al., "Arachidonic, Eicosapentaenoic, and Biosynthetically Related Fatty Acids in the Seed Lipids from a Primitive Gymnosperm, <i>Agathis robusta</i> ", <i>Lipids</i> , 1999, Vol. 34, No. 10, pp. 1083-1097.	
	CD	HONG, H., et al., "High-Level Production of γ -Linolenic Acid in <i>Brassica juncea</i> Using a $\Delta 6$ Desaturase from <i>Pythium irregulare</i> ", <i>Plant Physiology</i> , 2002, Vol. 129, pp. 354-362.	
	CE	ABBADI, A., et al., "Transgenic Oilseeds as Sustainable Source of Nutritionally Relevant C20 and C22 Polyunsaturated Fatty Acids?", <i>Eur. J. Lipid Sci. Technol.</i> , 2001, Vol. 103, pp. 106-113.	
	CF	SAYANOVA, O. V., et al., "Eicosapentaenoic Acid: Biosynthetic Routes and the Potential for Synthesis in Transgenic Plants", <i>Phytochemistry</i> , 2004, Vol. 65, pp. 147-158.	
	CG	HEINZ, E., "Docosahexaenoic Acid (DHA) in Transgenic Oilseeds: Which Approach Will Be Successful First?", <i>European Journal of Lipid Science and Technology</i> , 2002, Vol. 104, pp. 1-2.	
	CH	LUI, J.-W., et al., "Evaluation of the Seed Oils from a Canola Plant Genetically Transformed to Produce High Levels of γ -Linolenic Acid", Chapter 7 in " γ -Linolenic acid: Recent Advances in Biotechnology and Clinical Applications", Eds. Huang and Ziboh, AOCS Press, Champaign, Illinois, 2001, pp. 61-71.	
	CI	DERELLE, E., et al., "DNA Libraries for Sequencing the Genome of <i>Ostreococcus tauri</i> (Chlorophyta, Prasinophyceae): The Smallest Free-Living Eukaryotic Cell", <i>J. Phycol.</i> , 2002, Vol. 38, pp. 1150-1156.	
	CJ	DERELLE, E., et al., "Genome Analysis of the Smallest Free-Living Eukaryote <i>Ostreococcus tauri</i> Unveils Many Unique Features", <i>PNAS</i> , 2006, Vol. 103, No. 31, pp. 11647-11652.	
	CK	RAL, J.-P., et al., "Starch Division and Partitioning. A Mechanism for Granule Propagation and Maintenance in the Picophytoplanktonic Green Alga <i>Ostreococcus tauri</i> ", <i>Plant Physiology</i> , 2004, Vol. 136, pp. 3333-3340.	
	CL	" <i>Ostreococcus tauri</i> Delta-6-Desaturase (d6) Gene, Complete cds", Database EMBL Accession No. AY746357, July 8, 2005.	
	CM	SAYANOVA, O. V., et al., "Identification of <i>Primula</i> Fatty Acid Δ^6 -Desaturases with <i>n</i> -3 Substrate Preferences", <i>FEBS Letters</i> , 2003, Vol. 542, pp. 100-104.	

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				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	
Sheet	3	of	4	Attorney Docket Number	074017-0013-01-US	

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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 <i>Caenorhabditis elegans</i> ", Biochemical Society Transactions, 2000, Vol. 28, pp. 661-663.	
	CO	PARKER-BARNES, J. M., et al., "Identification and Characterization of an Enzyme Involved in the Elongation of n-6 and n-3 Polyunsaturated Fatty Acids", PNAS, Vol. 97, 2000, Vol. 97, No. 15, pp. 8284-8289.	
	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.	
	CR	INAGAKI, K., et al., "Identification and Expression of a Rat Fatty Acid Elongase Involved in the Biosynthesis of C18 Fatty Acids", Biosci. Biotechnol. Biochem., 2002, Vol. 66, No. 3, pp. 613-621.	
	CS	WALLIS, J. G., et al., "The Δ^8 -Desaturase of <i>Euglena gracilis</i> : An Alternate Pathway for Synthesis of 20-Carbon Polyunsaturated Fatty Acids", Archives of Biochemistry and Biophysics, 1999, Vol. 365, No. 2, pp. 307-316.	
	CT	QI, B., et al., "Identification of a cDNA Encoding a Novel C18- Δ^9 Polyunsaturated Fatty Acid-Specific Elongating Activity From the Docosahexaenoic Acid (DHA)-Producing Microalga, <i>Isochrysis galbana</i> ", FEBS Letters, 2002, Vol. 510, pp. 159-165.	
	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.	
	CV	KNUTZON, D. S., et al., "Identification of Δ^5 -Desaturase from <i>Mortierella alpina</i> by Heterologous Expression in Bakers' Yeast and Canola", The Journal of Biological Chemistry, 1998, Vol. 273, No. 45, pp. 29360-29366.	
	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 Δ^5 FA Desaturase from <i>Pythium irregulare</i> by Heterologous Expression in <i>Saccharomyces cerevisiae</i> and Oilseed Crops", Lipids, 2002, Vol. 37, No. 9, pp. 863-868.	
	CY	LEONARD, A. E., et al., "Cloning of a Human cDNA Encoding a Novel Enzyme Involved in the Elongation of Long-Chain Polyunsaturated Fatty Acids", Biochem. J., 2000, Vol. 350, pp. 765-770.	
	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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				Filing Date	September 6, 2016	
				First Named Inventor	Petra CIRPUS	
				Art Unit	1652	
				Examiner Name	Hope A. Robinson	
Sheet	4	of	4	Attorney Docket Number	074017-0013-01-US	

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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", <i>Marine Biotechnology</i> , 2004, Vol. 6, pp. 251-261.	
	CB1	ARMBRUST, E. V., et al., "The Genome of the Diatom <i>Thalassiosira pseudonana</i> : Ecology, Evolution, and Metabolism", <i>Science</i> , 2004, Vol. 306, pp. 79-86.	
	CC1	CLOUGH, S. J., et al., "Floral Dip: A Simplified Method for Agrobacterium-Mediated Transformation of <i>Arabidopsis thaliana</i> ", <i>The Plant Journal</i> , 1998, Vol. 16, No. 6, pp. 735-743.	
	CD1	QIU, X., et al., "Identification of a $\Delta 4$ Fatty Acid Desaturase from <i>Thraustochytrium</i> sp. Involved in the Biosynthesis of Docosahexanoic Acid by Heterologous Expression in <i>Saccharomyces cerevisiae</i> and <i>Brassica juncea</i> ", <i>The Journal of Biological Chemistry</i> , 2001, Vol. 276, No. 34, pp. 31561-31566.	
	AE1	MILLAR, A. A., et al., "Accumulation of Very-Long-Chain Fatty Acids in Membrane Glycerolipids Is Associated with Dramatic Alternations in Plant Morphology", <i>The Plant Cell</i> , 1998, Vol. 11, pp. 1889-1902.	
	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 <i>Ostreococcus tauri</i> ", <i>Biochem. J.</i> , 2005, Vol. 389, pp. 483-490.	
	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", <i>Progress in Lipid Research</i> , 2010, Vol. 49, pp. 108-119.	
	AH1	MEYER, A., et al., "Biosynthesis of Docosahexaenoic Acid in <i>Euglena gracilis</i> : Biochemical and Molecular Evidence for the Involvement of a $\Delta 4$ -Fatty Acyl Group Desaturase", <i>Biochemistry</i> , 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", <i>The Journal of Biological Chemistry</i> , 2003, Vol. 278, No. 37, pp. 35115-35126.	
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	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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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
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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.

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

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Attorney for Applicant

#87,380,869



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

Table with columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO., EXAMINER, ART UNIT, PAPER NUMBER, NOTIFICATION DATE, DELIVERY MODE. Includes application details for Petra Cirpus and examiner ROBINSON, HOPE A.

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

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.

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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

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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

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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

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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).

Application/Control Number: 15/256,914
Art Unit: 1652

Page 8

/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

Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Includes details for application 15/256,914, inventor Petra Cirpus, and attorney Drinker Biddle & Reath LLP.

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. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 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.

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 ω 3-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 ω 3-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 $\Delta 6$ -desaturase, a nucleic acid encoding a $\Delta 5$ -desaturase, a nucleic acid encoding a $\Delta 6$ -elongase, a nucleic acid encoding a $\omega 3$ -desaturase, a nucleic acid encoding a $\Delta 5$ -elongase, and a nucleic acid encoding a $\Delta 4$ -desaturase, wherein said nucleic acid encoding a $\Delta 5$ -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 $\Delta 5$ -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 $\Delta 5$ -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.

Registration No.: 57,209

DRINKER BIDDLE LLP

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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
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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:

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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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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/256,914	Filing Date 09/06/2016	<input type="checkbox"/> To be Mailed
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ENTITY: LARGE SMALL MICRO

APPLICATION AS FILED – PART I

FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)
<input type="checkbox"/> BASIC FEE <small>(37 CFR 1.16(a), (b), or (c))</small>	N/A	N/A	N/A	
<input type="checkbox"/> SEARCH FEE <small>(37 CFR 1.16(k), (l), or (m))</small>	N/A	N/A	N/A	
<input type="checkbox"/> EXAMINATION FEE <small>(37 CFR 1.16(o), (p), or (q))</small>	N/A	N/A	N/A	
TOTAL CLAIMS <small>(37 CFR 1.16(i))</small>	minus 20 =	*	X \$ =	
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>	minus 3 =	*	X \$ =	
<input type="checkbox"/> APPLICATION SIZE FEE <small>(37 CFR 1.16(s))</small>	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).			
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT <small>(37 CFR 1.16(j))</small>				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

	(Column 1)	(Column 2)	(Column 3)	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT	03/23/2017	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR			
	Total <small>(37 CFR 1.16(i))</small>	* 23	Minus	** 22	= 1	X \$80 = 80
	Independent <small>(37 CFR 1.16(h))</small>	* 2	Minus	***3	= 0	X \$420 = 0
	<input type="checkbox"/> Application Size Fee <small>(37 CFR 1.16(s))</small>					
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <small>(37 CFR 1.16(j))</small>						
					TOTAL ADD'L FEE	80

	(Column 1)	(Column 2)	(Column 3)	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR			
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	X \$ =
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	X \$ =
	<input type="checkbox"/> Application Size Fee <small>(37 CFR 1.16(s))</small>					
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <small>(37 CFR 1.16(j))</small>						
					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".

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KIMBERLY WHITE

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.**

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Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Includes details for application 15/256,914, inventor Petra Cirpus, and examiner ROBINSON, HOPE A.

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

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

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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

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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%

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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.

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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

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NON-PATENT DOCUMENTS

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*	Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)				
U					
V					
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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.

Substitute for form 1449/PTO <h2 style="text-align: center;">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</h2> <p style="text-align: center;">(Use as many sheets as necessary)</p>				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
Sheet	1	of	4		

U. S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)				
	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		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
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)					
	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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			Examiner Name	Hope A. Robinson
			Attorney Docket Number	074017-0013-01-US
Sheet	2	of	4	

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 <i>Arabidopsis</i> to Produce Nutritionally Important DHA in Seed Oil", <i>Functional Plant Biology</i> , 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.	
	CC	WOLFF, R. L., et al., "Arachidonic, Eicosapentaenoic, and Biosynthetically Related Fatty Acids in the Seed Lipids from a Primitive Gymnosperm, <i>Agathis robusta</i> ", <i>Lipids</i> , 1999, Vol. 34, No. 10, pp. 1083-1097.	
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	CE	ABBADI, A., et al., "Transgenic Oilseeds as Sustainable Source of Nutritionally Relevant C20 and C22 Polyunsaturated Fatty Acids?", <i>Eur. J. Lipid Sci. Technol.</i> , 2001, Vol. 103, pp. 106-113.	
	CF	SAYANOVA, O. V., et al., "Eicosapentaenoic Acid: Biosynthetic Routes and the Potential for Synthesis in Transgenic Plants", <i>Phytochemistry</i> , 2004, Vol. 65, pp. 147-158.	
	CG	HEINZ, E., "Docosahexaenoic Acid (DHA) in Transgenic Oilseeds: Which Approach Will Be Successful First?", <i>European Journal of Lipid Science and Technology</i> , 2002, Vol. 104, pp. 1-2.	
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	CI	DERELLE, E., et al., "DNA Libraries for Sequencing the Genome of <i>Ostreococcus tauri</i> (Chlorophyta, Prasinophyceae): The Smallest Free-Living Eukaryotic Cell", <i>J. Phycol.</i> , 2002, Vol. 38, pp. 1150-1156.	
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	CK	RAL, J.-P., et al., "Starch Division and Partitioning. A Mechanism for Granule Propagation and Maintenance in the Picophytoplanktonic Green Alga <i>Ostreococcus tauri</i> ", <i>Plant Physiology</i> , 2004, Vol. 136, pp. 3333-3340.	
	CL	" <i>Ostreococcus tauri</i> Delta-6-Desaturase (d6) Gene, Complete cds", Database EMBL Accession No. AY746357, July 8, 2005.	
	CM	SAYANOVA, O. V., et al., "Identification of <i>Primula</i> Fatty Acid Δ^6 -Desaturases with <i>n</i> -3 Substrate Preferences", <i>FEBS Letters</i> , 2003, Vol. 542, pp. 100-104.	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	06/24/2017
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*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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Sheet	3	of	4		

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 <i>Caenorhabditis elegans</i> ", 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.	
	CR	INAGAKI, K., et al., "Identification and Expression of a Rat Fatty Acid Elongase Involved in the Biosynthesis of C18 Fatty Acids", Biosci. Biotechnol. Biochem., 2002, Vol. 66, No. 3, pp. 613-621.	
	CS	WALLIS, J. G., et al., "The Δ^8 -Desaturase of <i>Euglena gracilis</i> : An Alternate Pathway for Synthesis of 20-Carbon Polyunsaturated Fatty Acids", Archives of Biochemistry and Biophysics, 1999, Vol. 365, No. 2, pp. 307-316.	
	CT	QI, B., et al., "Identification of a cDNA Encoding a Novel C18- Δ^9 Polyunsaturated Fatty Acid-Specific Elongating Activity From the Docosahexaenoic Acid (DHA)-Producing Microalga, <i>Isochrysis galbana</i> ", FEBS Letters, 2002, Vol. 510, pp. 159-165.	
	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.	
	CV	KNUTZON, D. S., et al., "Identification of Δ^5 -Desaturase from <i>Mortierella alpina</i> by Heterologous Expression in Bakers' Yeast and Canola", The Journal of Biological Chemistry, 1998, Vol. 273, No. 45, pp. 29360-29366.	
	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 Δ^5 FA Desaturase from <i>Pythium irregulare</i> by Heterologous Expression in <i>Saccharomyces cerevisiae</i> and Oilseed Crops", Lipids, 2002, Vol. 37, No. 9, pp. 863-868.	
	CY	LEONARD, A. E., et al., "Cloning of a Human cDNA Encoding a Novel Enzyme Involved in the Elongation of Long-Chain Polyunsaturated Fatty Acids", Biochem. J., 2000, Vol. 350, pp. 765-770.	
	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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Substitute for form 1449/PTO <h2 style="text-align: center;">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</h2> <p style="text-align: center;">(Use as many sheets as necessary)</p>				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
Sheet	4	of	4		

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", <i>Marine Biotechnology</i> , 2004, Vol. 6, pp. 251-261.	
	CB1	ARMBRUST, E. V., et al., "The Genome of the Diatom <i>Thalassiosira pseudonana</i> : Ecology, Evolution, and Metabolism", <i>Science</i> , 2004, Vol. 306, pp. 79-86.	
	CC1	CLOUGH, S. J., et al., "Floral Dip: A Simplified Method for Agrobacterium-Mediated Transformation of <i>Arabidopsis thaliana</i> ", <i>The Plant Journal</i> , 1998, Vol. 16, No. 6, pp. 735-743.	
	CD1	QIU, X., et al., "Identification of a $\Delta 4$ Fatty Acid Desaturase from <i>Thraustochytrium</i> sp. Involved in the Biosynthesis of Docosahexanoic Acid by Heterologous Expression in <i>Saccharomyces cerevisiae</i> and <i>Brassica juncea</i> ", <i>The Journal of Biological Chemistry</i> , 2001, Vol. 276, No. 34, pp. 31561-31566.	
	AE1	MILLAR, A. A., et al., "Accumulation of Very-Long-Chain Fatty Acids in Membrane Glycerolipids Is Associated with Dramatic Alternations in Plant Morphology", <i>The Plant Cell</i> , 1998, Vol. 11, pp. 1889-1902.	
	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 <i>Ostreococcus tauri</i> ", <i>Biochem. J.</i> , 2005, Vol. 389, pp. 483-490.	
	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", <i>Progress in Lipid Research</i> , 2010, Vol. 49, pp. 108-119.	
	AH1	MEYER, A., et al., "Biosynthesis of Docosahexaenoic Acid in <i>Euglena gracilis</i> : Biochemical and Molecular Evidence for the Involvement of a $\Delta 4$ -Fatty Acyl Group Desaturase", <i>Biochemistry</i> , 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", <i>The Journal of Biological Chemistry</i> , 2003, Vol. 278, No. 37, pp. 35115-35126.	
	AJ1	GUNSTONE, F. D., "Vegetable Oils", In: <i>Bailey's Industrial Oil and Fat Products</i> , 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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		Application Number	Not Yet Assigned
		Filing Date	Concurrent Herewith
		First Named Inventor	Petra Cirpus
		Art Unit	N/A
		Examiner Name	Not Yet Assigned
Sheet	1	of	6
		Attorney Docket Number	074017-0013-01-US

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
	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	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
		Country Code ³ -Number ⁴ - Kind Code ⁵ (if known)				
	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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INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Application Number	Not Yet Assigned
				Filing Date	Concurrent Herewith
				First Named Inventor	Petra Cirpus
				Art Unit	N/A
				Examiner Name	Not Yet Assigned
Sheet	2	of	6	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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			Examiner Name	Not Yet Assigned	
Sheet	3	of	6	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, <i>Synechococcus</i> sp.", Lipids, 35:10 (2000), pp. 1061-1064.	
	CF**	Magnuson, K. et al., "Regulation of Fatty Acid Biosynthesis in <i>Escherichia coli</i> ", 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 <i>Porphyridium Cruentum</i> ", 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 <i>Phaeodactylum tricornutum</i> Front-End Desaturases Involved in Eicosapentaenoic Acid Biosynthesis", Eur. J. Biochem. 269 (2002), pp. 4105-4113.	
	CM**	Totani, N. et al., "The Filamentous Fungus <i>Mortierella alpina</i> , High in Arachidonic 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**	Tvrdek, 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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		Art Unit	N/A
		Examiner Name	Not Yet Assigned
Sheet	4	of	6
		Attorney Docket Number	074017-0013-01-US

CS**	Sakuradani, E. et al., " $\Delta 6$ -Fatty Acid Desaturase from an Arachidonic Acid-Producing <i>Mortierella</i> Fungus Gene Cloning and Its Heterologous Expression in a Fungus, <i>Aspergillus</i> ", Gene 238 (1999), pp. 445-453.
CT**	Kinney, A.J., "Genetic Engineering 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 <i>OLE1</i> Gene of <i>Saccharomyces cerevisiae</i> Encodes the $\Delta 9$ Fatty Acid Desaturase and Can Be Functionally Replaced by the Rat Stearoyl-CoA Desaturase Gene", The Journal 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 $\Delta 12$ - and $\Delta 6$ -Desaturases from <i>Mortierella alpina</i> and Recombinant Production of γ -Linolenic Acid in <i>Saccharomyces cerevisiae</i> ", 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 <i>Shewanella</i> sp. in a Transgenic Marine Cyanobacterium, <i>Synechococcus</i> 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 <i>Brassica napus</i> ", 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 $\Delta 6$ -polyunsaturated Fatty Acids from the Moss <i>Physcomitrella patens</i> ", The Plant Journal 31:3 (2002), pp. 255-268.
CG1**	Millar, A.A. et al., " <i>CUT1</i> , 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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		Art Unit	N/A
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Sheet	5	of	6
		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**	Ohlogge, 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 <i>Phytophthora infestans</i> 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**	Abadi, 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 <i>Phytophthora infestans</i> cDNA, mRNA sequence." Database EMBL, Accession No. BE777235, September 21, 2000.
CQ1**	Domergue, F. et al., "Cloning and Functional Characterization of <i>Phaeodactylum tricornutum</i> 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 <i>Phytophthora infestans</i> 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 <i>Porphyridium cruentum</i> ", Plant Physiol. 114 (1997), pp. 223-230.
CT1**	Pereira, S.L. et al., "A Novel ω 3-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 <i>Arabidopsis</i> ", Proc. Natl. Acad. Sci. USA 94 (1997), pp. 1142-1147.
CW1**	KAJIKAWA, M., et al., "Isolation and Functional Characterization of Fatty Acid Δ 5-Elongase Gene from the Liverwort <i>Marchantia polymorpha</i> L.", FEBS Letters, 2006, Vol. 580, pp. 149-154.
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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
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*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.

¹Applicant's unique citation designation number (optional). ²Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH (A, B)

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Substitute for form 1449A/B/PTO		Complete if Known			
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)		Application Number	Not Yet Assigned		
		Filing Date	Concurrent Herewith		
		First Named Inventor	Petra Cirpus		
		Art Unit	N/A		
		Examiner Name	Not Yet Assigned		
Sheet	6	of	6	Attorney Docket Number	074017-0013-01-US

CC2**	DOMERGUE, F., et al., "New Insight into <i>Phaeodactylum tricornutum</i> Fatty Acid Metabolism. Cloning and Functional Characterization of Plastidial and Microsomal Δ 12-Fatty Acid Desaturases", <i>Plant Physiology</i> , 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", <i>Nature Biotechnology</i> , 2005, Vol. 23, pp. 1013-1017.
CE2**	NAKAMURA, M. T., et al., "Structure, Function, and Dietary Regulation of Δ 6, Δ 5, and Δ 9 Desaturases", <i>Annu. Rev. Nutr.</i> , 2004, Vol. 24, pp. 345-376.
CF2**	" <i>P. patens</i> Delta6 Elongase SEQ ID 29", <i>GeneSeq Database Accession No. ABG73608</i> , March 25, 2003.
CG2**	"Subname: Full = Polyunsaturated Fatty Acid Elongase elvol5a", <i>UniProt Database Accession No. Q8AWE7</i> , October 25, 2005.
CH2**	"Polyunsaturated Fatty Acid Elongase (ELOVL Family Member 5, Elongation of Long Chain Fatty Acids) (YEAST)", <i>UniProt Database Accession No. Q8AX86</i> , March 1, 2003.
CI2**	"633167 NCCWA 1RT <i>Oncorhynchus Mykiss</i> cDNA Clone 1RT126D03_B_B02 5', mRNA Sequence", <i>EMBL Database Accession No. CA360014</i> , November 7, 2002.
CJ2**	HUANG, Y.-S., et al., "Enzymes for Transgenic Biosynthesis of Long-Chain Polyunsaturated Fatty Acids", <i>Biochimie</i> , 2004, Vol. 86, No. 11, pp. 793-798.
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CV2**	Qi, B., et al., "Production of Very Long Chain Polyunsaturated Omega-3 and Omega-6 Fatty Acids in Plants", <i>Nature Biotechnology</i> , 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", <i>Marine Biotechnology</i> , 2006, 8: 103-109.

Examiner Signature	/HOPE A ROBINSON/	Date Considered	06/24/2017
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*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.

¹Applicant's unique citation designation number (optional). ²Applicant is to place a check mark here if English language Translation is attached.

Search Notes 	Application/Control No. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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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BIB DATA SHEET

CONFIRMATION NO. 4050

SERIAL NUMBER 15/256,914	FILING or 371(c) DATE 09/06/2016 RULE	CLASS 514	GROUP ART UNIT 1652	ATTORNEY DOCKET NO. 074017-0013-01-US	
APPLICANTS BASF Plant Science GmbH, Ludwigshafen, GERMANY; INVENTORS 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; ** 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 <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 35 USC 119(a-d) conditions met <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No Verified and Acknowledged <u>/HOPE A ROBINSON/</u> Examiner's Signature	<input type="checkbox"/> Met after Allowance Initials	STATE OR COUNTRY GERMANY	SHEETS DRAWINGS 0	TOTAL CLAIMS 22	INDEPENDENT CLAIMS 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:		<input type="checkbox"/> All Fees <input type="checkbox"/> 1.16 Fees (Filing) <input type="checkbox"/> 1.17 Fees (Processing Ext. of time) <input type="checkbox"/> 1.18 Fees (Issue) <input type="checkbox"/> Other _____ <input type="checkbox"/> Credit		

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

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.jp/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelchon (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 ω 3-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 ω 3-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 $\Delta 6$ -desaturase, a nucleic acid encoding a $\Delta 5$ -desaturase, a nucleic acid encoding a $\Delta 6$ -elongase, a nucleic acid encoding a $\omega 3$ -desaturase, a nucleic acid encoding a $\Delta 5$ -elongase, and a nucleic acid encoding a $\Delta 4$ -desaturase, wherein said nucleic acid encoding a $\Delta 5$ -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 $\Delta 5$ -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 $\Delta 5$ -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 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.

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 ω 3 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 ω 3 fatty acids is only 14.58 to 15.72 %. Thus, WO 2005/083093 clearly does not disclose that the content of polyunsaturated ω 3 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 ω 3 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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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.

10 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.

15 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 $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity, where the nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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.

20
25 In a preferred embodiment there is additionally provision of further nucleic acid sequences which code for a polypeptide having the activity of an $\omega 3$ -desaturase and/or of a $\Delta 4$ -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
5 one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity and which is modified by comparison with the nucleic acid
10 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
15 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
20 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
25 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 Prokaryotes*. Thieme: Stuttgart, New York, and the references therein, and
30 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.

5 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).

10 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 Engineering*, 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.* 15 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.

20 Depending on the desaturation pattern, two large classes of polyunsaturated fatty acids, the $\omega 6$ and the $\omega 3$ 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).

25 The fatty acid linoleic acid ($18:2^{\Delta 9,12}$) acts as starting material for the $\omega 6$ metabolic pathway, while the $\omega 3$ pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed from linoleic acid by the activity of an $\omega 3$ -desaturase (Tocher et al. (1998) *Prog. Lipid Res.* 37: 73-117; Domergue et al. (2002) *Eur. J. Biochem.* 269: 4105-4113).

30 Mammals, and thus also humans, have no corresponding desaturase activity ($\Delta 12$ - and $\omega 3$ -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^{Δ5,8,11,14}), an ω6-fatty acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, 20:5^{Δ5,8,11,14,17}) and docosahexaenoic acid (DHA, 22:6^{Δ4,7,10,13,17,19}) are synthesized via a sequence of desaturase and elongase reactions.

- 5 The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C₂₀- and C₂₂-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 dehydration step (dehydratase) and a final reduction step
10 (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
15 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
20 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
25 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
30 arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG₂ series) which are formed from the ω6-fatty acids, generally promote inflammatory reactions, while eicosanoids (known as the PG₃ series) from ω3-fatty acids have little or no proinflammatory effect.

Polyunsaturated long-chain ω 3-fatty acids such as eicosapentaenoic acid (= EPA, C20:5 ^{Δ 5,8,11,14,17}) or docosahexaenoic acid (= DHA, C22:6 ^{Δ 4,7,10,13,16,19}) 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 ω 3-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 ^{Δ 4,7,10,13,16,19}) or eicosapentaenoic acid (= EPA, C20:5 ^{Δ 5,8,11,14,17}) 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 *Cryptocodinium* 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 ^{Δ 5,8,11,14}), dihomo- γ -linolenic acid (DHGL, C20:3 ^{Δ 8,11,14}) or docosapentaenoic acid (DPA, C22:5 ^{Δ 7,10,13,16,19}) 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 Δ 9-desaturase. WO 93/11245 claims a Δ 15-desaturase and WO 94/11516 a Δ 12-desaturase. Further desaturases 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).

5 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. $\Delta 6$ -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
10 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,
15 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
20 in plants (C₂₈-C₃₂). 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
25 *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.
30 (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
5 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
10 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
15 oilseeds, genes which encode enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. These genes encode for example Δ 6-desaturases, Δ 6-elongases, Δ 5-desaturases or Δ 4-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 Δ 6-desaturase
20 genes from the moss *Physcomitrella patens* and Δ 6-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).
25 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
30 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.

- 5 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 $\Delta 5$ -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
10 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
15 transgenic plant, comprising the provision in the plant of at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity,

20 where the nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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 $\Delta 4$ -desaturase activity in the plant.

25 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 $\Delta 6$ -desaturase activity, a polypeptide having a $\Delta 6$ -elongase activity, a polypeptide having a $\Delta 5$ -desaturase activity and a polypeptide having a $\Delta 5$ -elongase activity are present together in one plant. The "provision in the plant" thus comprises the introduction of the nucleic acid sequences
30 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.

5 The nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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.

10 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
15 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
20 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
25 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
30 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.jp/Kodon/E.html. In addition, several companies provide software for gene optimization, such as, for example, Entelechon (Software Leto) or Geneart (Software
5 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
10 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.

15 It is particularly preferred for a nucleic acid sequence from *Ostreococcus tauri* which codes for a polypeptide having a $\Delta 5$ -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
20 $\Delta 5$ -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
25 sequences which code for a polypeptide having the activity of a $\Delta 5$ -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 $\Delta 6$ -desaturase activity is preferably selected from the group consisting of:

30 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 NO: 2,

5 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
10 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.

15 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
20 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 $\Delta 6$ -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,

25 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
30 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

5 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 $\Delta 6$ -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 $\Delta 5$ -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 Δ 4-desaturase are introduced into the plant.

5 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,
- 10 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
15 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 (= 20 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 Δ 4-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, 25 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 $\Delta 4$ -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 $\Delta 6$ -desaturase activity, a $\Delta 6$ -elongase activity, a $\Delta 5$ -desaturase activity and a $\Delta 5$ -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 $\Delta 4$ -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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and the $\Delta 5$ -elongase, and if appropriate the $\omega 3$ -desaturase and/or the $\Delta 4$ -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, bulbils 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. 5 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.

10 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).

20 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 25 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. 30

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 5 (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 10 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 $\Delta 5$ -elongase and which is 15 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 20 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 25 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 $\Delta 5$ -elongase and which is modified by comparison with the nucleic acid sequence in the organism from which 30 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, 5 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 10 sequences which codes for a polypeptide having the activity of a $\Delta 4$ -desaturase activity are required. A nucleic acid sequence which codes for a polypeptide having the activity of a $\Delta 4$ -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.

15 In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a $\Delta 5$ -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 20 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, 25 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 30 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, monoacylglycerides 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 C₂₀ or C₂₂ 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 C₁₈-, C₂₀- and/or C₂₂-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), puniic 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^{Δ3,8,12,15,18,21}).

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
5 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
10 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
15 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,
20 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*
25 [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 annuus* (sunflower), *Lactuca sativa*,
30 *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* (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*,
5 *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*,
10 *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 sativa* (hemp), Convolvulaceae such as the genera *Ipomoea*, *Convolvulus*, e.g. the genera and species *Ipomoea batatas*, *Ipomoea pandurata*, *Convolvulus*
15 *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 *Cucurbita*, e.g. the genera
20 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
25 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
30 *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*, *Pseudalbizia 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*
5 *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*
10 *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*
15 *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), Lythraeae such as the genus *Punica*, e.g. the
20 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
25 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*,
30 *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 viciifolia*), Egyptian clover (*Trifolium alexandrinum*) 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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity, advantageously in combination with nucleic acid sequences which code for polypeptides having $\omega 3$ -desaturase and/or $\Delta 4$ -desaturase activity, and further nucleic acid sequences which code for polypeptides of fatty acid or lipid metabolism, such as further polypeptides having $\Delta 5$ -, $\Delta 6$ -, $\Delta 8$ -, $\Delta 12$ -desaturase or $\Delta 5$ -, $\Delta 6$ - or $\Delta 9$ -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^{Δ9,12}), 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^{Δ9,12,15}), 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 Δ 6-elongase, Δ 6-desaturase, Δ 5-desaturase and/or Δ 6-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 Δ 12-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 Δ 12-

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.

5 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
10 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
15 such as algae, for example algae of the family of Prasinophyceae such as from the genera *Heteromastix*, *Mammella*, *Mantoniella*, *Micromonas*, *Nephroselmis*, *Ostreococcus*, *Prasinocladus*, *Prasinococcus*, *Pseudoscourfieldia*, *Pycnococcus*, *Pyramimonas*, *Scherffelia* or *Tetraselmis* such as the genera and species *Heteromastix longifillis*, *Mamiella gilva*, *Mantoiella squamata*, *Micromonas pusilla*, *Nephroselmis olivacea*, *Nephroselmis pyriformis*,
20 *Neproselmis rotunda*, *Ostreococcus tauri*, *Ostreococcus* sp. *Prasinocladus ascus*, *Prasinocladus lubricus*, *Pycnococcus provasolii*, *Pyramimonas amyliifera*, *Pyramimonas disomata*, *Pyramimonas obovata*, *Pyramimonas orientalis*, *Pyramimonas parkae*, *Pyramimonas spinefera*, *Pyramimonas* sp., *Tetraselmis apiculata*, *Tetraselmis carteriaformis*, *Tetraselmis chui*, *Tetraselmis convolutae*, *Tetraselmis desikacharyi*, *Tetraselmis gracilis*,
25 *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 Euglenaceae such as from the genera *Ascoglena*, *Astasia*, *Colacium*, *Cyclidiopsis*, *Euglena*, *Euglenopsis*,
30 *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 *Cryptocodinium*, algae/diatoms such as *Thalassiosira* or *Phaeodactylum*, mosses such as *Physcomitrella* or *Ceratodon* or
5 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
10 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*,
15 *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
20 algae such as the genera *Mantoniella* or *Ostreococcus* or from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum* or from algae such as *Cryptocodinium*.

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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase and, if
25 appropriate, nucleic acid sequences which code for an $\omega 3$ -desaturase and/or a $\Delta 4$ -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
30 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.

5 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
- 15 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -elongase activity, advantageously in combination with nucleic acid sequences which encode proteins having $\omega 3$ -desaturase and/or $\Delta 4$ -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
5 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
10 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, cotyledons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for
15 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
20 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
25 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
30 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.

5 The PUFAs or LCPUFAs produced by this process are preferably C₂₀ and/or C₂₂ fatty acid molecules having at least four double bonds in the fatty acid molecule, preferably five or six double bonds. These C₂₀ and/or C₂₂ 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.

10 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.

15 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
20 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), chaulmoogric acid (cyclopentenedodecanoic acid),
25 furan fatty acid (9,12-epoxyoctadeca-9,11-dienonic acid), vernonic acid (9,10-epoxyoctadec-12-enonic acid), tarinic acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (11-octadecen-9-ynoic acid), 6,9-octadecenyonic acid, pyrulic acid (10-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-
30 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-
5 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%
10 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
15 (tetracosahexaenoic acid, C₂₃:6^{A3,8,12,15,18,21}).

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
20 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.

25 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
30 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
5 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
10 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
15 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, H₂SO₄. However, the fatty acids can also be liberated directly without the above-described processing steps.

20 Substrates of the nucleic acid sequences used in the process which encode polypeptides with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity and optionally nucleic acid sequences which encode polypeptides having $\omega 3$ -desaturase and/or $\Delta 4$ -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
25 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
30 acid elongase(s) are advantageously C₁₆-, C₁₈- or C₂₀-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 C₁₆-fatty acids and/or polyunsaturated C₁₈-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.

5 After one elongation cycle, this enzyme activity leads either starting from C₁₆-fatty acids to C₁₈-fatty acids or starting from C₁₈-fatty acids to C₂₀-fatty acids, and after two elongation cycles starting from C₁₆-fatty acids leads to C₂₀-fatty acids. The activity of the desaturases or elongases used in the process according to the invention preferably leads to C₂₀- and/or C₂₂-fatty acids, advantageously with at least two or three double bonds in the fatty acid molecule,

10 preferably with four, five or six double bonds, especially preferably to C₂₂-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 C₁₈-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

15 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

20 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

25 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

30 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.

5 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
10 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.

15 The nucleic acid construct may comprise more than one nucleic acid sequence coding for a polypeptide having the enzymatic activity of a $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, and/or $\omega 3$ -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 $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, and/or $\omega 3$ -desaturase to be present.

20 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
25 the stop codon. After the amplification, the amplicon 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 protocol (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
30 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, pBII101, 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity and, if appropriate, a $\Delta 4$ -desaturase and/or $\omega 3$ -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
5 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)
10 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
15 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 ω 3-desaturase, Δ 6-desaturase, Δ 6-elongase, Δ 5-elongase,
20 Δ 4-desaturase and/or Δ 5-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
25 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 *Caenorhabditis* or *Oncorhynchus* or plants such as algae or
30 mosses such as the genera *Shewanella*, *Physcomitrella*, *Thraustochytrium*, *Fusarium*, *Phytophthora*, *Ceratodon*, *Pytium irregulare*, *Mantoniella*, *Ostreococcus*, *Isochrysis*, *Aleurita*, *muscarioides*, *Mortierella*, *Borago*, *Phaeodactylum*, *Cryptocodium*, 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*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*,
5 *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 $\Delta 12$ -desaturase, $\Delta 9$ -elongase or $\Delta 8$ -desaturase. The nucleic acid sequences used in
10 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 $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 4$ -desaturase are functionally linked to one or more regulatory signals to increase the gene expression. These
15 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.
20 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
25 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
30 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
5 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
10 another in order to obtain progeny which contains all gene constructs 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,
15 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase or $\Delta 5$ -elongase and if appropriate the $\omega 3$ -desaturase or $\Delta 4$ -desaturase and
20 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
25 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
30 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
5 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
10 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 inducers, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a
15 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
20 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,
25 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, $\Delta 8$ -desaturase, $\Delta 9$ -desaturase, $\Delta 12$ -desaturase and/or $\Delta 9$ -elongase.

In this context, the abovementioned nucleic acids or genes can be cloned into expression
30 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
5 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
10 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 $\Delta 6$ -desaturates, $\Delta 6$ -elongase, $\Delta 5$ -desaturate and/or $\Delta 5$ -elongase genes can be expressed in bacterial cells, insect cells (using baculovirus expression vectors), yeast cells
15 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*
20 *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
25 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
30 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, λ gt11 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
5 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
10 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).

15 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,
20 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
25 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
30 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase or $\Delta 5$ -desaturase and, if appropriate, the $\omega 3$ -desaturase or $\Delta 4$ -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 $\Delta 5$ -elongase, $\omega 3$ -desaturase, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 4$ -desaturase or $\Delta 5$ -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 $\omega 3$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase, $\Delta 4$ -desaturase and/or $\Delta 6$ -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
5 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
10 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-
15 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
20 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
25 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
30 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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase and/or Δ 6-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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-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 ω 3-desaturase-, Δ 6-desaturase-, Δ 5-desaturase-, Δ 5-elongase-, Δ 4-desaturase- or Δ 6-elongase-encoding sequence, e.g. by saturation mutagenesis, and the resulting mutants can be screened for the ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-elongase activity described herein in order to identify mutants which have retained the ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-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:	
German application number	this subsequent	
102006008030.0	application	Organism

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

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
 5 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.

5 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 $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity. These are the following sequences:

Gene name	SEQ ID	Amino acids
OtELO1.1, ($\Delta 6$ -Elongase)	SEQ ID NO: 143	292
OtELO2.1, ($\Delta 5$ -Elongase)	SEQ ID NO: 109	300

10 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:

15 40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down and resuspended in 100 μ l 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
20 was carried out in each case with 1 μ l of thawed cells, 200 μ M dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 μ l. 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.

25 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 (Δ 6-elongase) and SEQ ID NO: 109 (coding for a protein identified by SEQ ID NO: 110) (Δ 5-elongase) were adapted to the codon usage in oilseed rape, flax and soybean. For this purpose, the amino acid sequence of the Δ 6-elongase and of the Δ 5-elongase (SEQ ID NO: 144 for the Δ 6-elongase; SEQ ID NO: 65 for the Δ 5-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 (Δ 5-elongase) and SEQ ID NO: 122 (coding for a protein identified by SEQ ID NO: 123) (Δ 6-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 Δ 6-elongase sequence) and pOTE2.2 (comprising the Δ 5-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, (Δ 6-elongase)	SEQ ID NO: 143	292
pOTE1.2, (Δ 6-elongase)	SEQ ID NO: 122	292, codon-optimized
pOTE2.1, (Δ 5-elongase)	SEQ ID NO: 109	300
pOTE2.2, (Δ 5-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 Δ 6-elongase and Δ 5-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 OD₆₀₀ of 5 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, 10 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

15 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 NaHCO₃, 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 20 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 NaHCO₃, pH 8.0 and with 2 ml of distilled water. The PE phases were then dried with Na₂SO₄, evaporated under argon 25 and taken up in 100 μ l of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 μ m, 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).

30 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 $\Delta 5$ -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 conditions 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:

Cn11 C 5': gaattcggcgcgccgagctcctcgcagcaacggtccggcggtatagagtgggtaattcga (SEQ ID NO: 200)

Cn11 C 3': cccgggatc gatccggcagatctccaccatttttgggtgtgat (SEQ ID NO: 201)

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l of Advantage polymerase (Clontech)

5 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

10

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.

15

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:

20

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

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

25 Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l of Advantage polymerase (Clontech)

PCR reaction conditions:

5 Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

10 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
15 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-Cn11-C_OCS was verified by sequencing.

In the next step, the Cn11-B promoter was amplified by PCR by means of the following primers:

20 Cn11-B 5': aggcctcaacgggtccggcggtatag (SEQ ID NO: 204)

Cn11-B 3': cccggggttaacgctagcgggcccgatatcggatcccatttttggtggtgattggttct (SEQ ID NO: 205)

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

25 5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

5 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 *Stu*I and
10 then for 12 hours at 25°C with the restriction enzyme *Sma*I. The vector pUC19-Cn11-C was
incubated for 12 hours at 25°C with the restriction enzyme *Sma*I. 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
15 product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The
resulting plasmid pUC19-Cn11-C_Cn11B_OCS was verified by sequencing.

In a further step, the OCS terminator for Cn11B was inserted. To this end, the PCR was
carried out using the following primers:

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

20 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 MgCl₂

25 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

5 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-
10 Cn11C_Cn11B_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
15 used for this purpose. The resulting plasmid pUC19-Cn11-C_Cn11B_OCS2 was verified by sequencing.

In the next step, the Cn11-A promoter is amplified by PCR using the following primers:

Cn11-B 5': aggcctcaacgggtccggcggtatagag (SEQ ID NO: 208)

Cn11-B 3': aggccttctagactgcaggcggccgcccgcatTTTTGGTGGTGATTGGT (SEQ ID NO: 209)

20

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

25 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

5 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.

15 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': aagcttggcgcgccgagctcgtcgcacggacaatcagtaaattgaacggaga (SEQ ID NO: 211)

20 Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

25 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

5

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.

15 In the next step, the plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the $\Delta 6$ -, $\Delta 5$ -desaturase and $\Delta 6$ -elongase. To this end, the *Phytium irregulare* $\Delta 6$ -desaturase (WO02/26946) was amplified using the following PCR primers:

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

20 D6Des(Pir) 3': ccatggccccgggttacatcgctgggaactcggtgat (SEQ ID NO: 213)

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

25 5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l 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

5 Number of cycles: 35

The PCR product was first incubated for 2 hours at 37°C with the restriction enzyme Bg/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
10 Bg/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-
15 Cnl1_d6Des(Pir) was verified by sequencing.

In the next step, the plasmid pUC19-Cnl1_d6Des(Pir) was used for cloning the *Thraustochytrium* ssp. $\Delta 5$ -desaturase (WO02/26946). To this end, the *Thraustochytrium* ssp. $\Delta 5$ -desaturase was amplified using the following PCR primers:

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

20 D5Des(Tc) 3': ggcgccgacaccaagaagcaggactgagatc (SEQ ID NO: 215)

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

25 5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l 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

5 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 *EcoRV*. The vector pUC19-Cnl1_d6Des(Pir) was incubated for 2 hours at 37°C with the restriction enzyme BamHI and
10 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-
15 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* $\Delta 6$ -elongase (WO01/59128), for which purpose the latter was amplified using the following PCR primers:

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

20 D6Elo(Pp) 3': gcaaaggagctaaaactgagtgatctaga (SEQ ID NO: 217)

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

25 5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l 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

5 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
10 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-
15 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
20 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-
25 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)

30 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 MgCl₂
- 5 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:

- 10 Annealing temperature: 1 min 55°C
- Denaturation temperature: 1 min 94°C
- Elongation temperature: 2 min 72°C
- Number of cycles: 35

- 15 The PCR product was incubated for 2 hours at 37°C with the restriction enzyme Sall. The vector pUC19 was incubated for 2 hours at 37°C with the restriction enzyme Sall. 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
- 20 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)
- 25 D12Des(Co) 3': ccatggttaaactcttattacgatacc (SEQ ID NO: 221)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

5 0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

10 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 Bg/II and thereafter for 2 hours at the same temperature with NcoI. The vector pUC19-Cnl1_OCS was
15 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
20 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 Sall. Thereafter, the vector fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised.
25 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-Cn11_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co). To this end, pUC19-Cn11_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) was incubated for 2 hours at 37°C with the restriction enzyme *AscI*. The vector pGPTV was treated in the same manner.

5 Thereafter, the fragment from pUC19-Cn11_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

10 resulting plasmid pGPTV- Cn11_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.

15 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:

20 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.

25 In the second step, the expression cassette was cut out of pUC19-Cn11_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

30 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.

10 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 metabolites 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 $\Delta 5$ -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 Sall/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 $\Delta 6$ -desaturase (SEQ ID NO: 1), of the $\Delta 5$ -desaturase (SEQ ID NO: 51) and $\Delta 6$ -elongase (SEQ ID NO: 171) sequences
5 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 Sall, the plasmid pUC-D6 was digested with Sall/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6 was then digested with Sall, the plasmid pUC-D5 was
10 digested with Sall/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6-D5 was then digested once more with Sall, the plasmid pUC-E6(Tc) with Sall/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
15 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 $\omega 3$ Des (SEQ ID NO: 193) was cloned into pUC19-35S. The resulting plasmid pUC- $\omega 3$ Pi was transferred via Sall/XhoI into the binary vectors pGPTV-D6D5E6(Tc) and pGPTV-D6D5E6(Tp). The resulting vectors pGPTV-
20 D6D5E6(Tc) $\omega 3$ Pi and pGPTV-D6D5E6(Tp) $\omega 3$ Pi were employed for the plant transformation.

In a further procedure, the open reading frame of the optimized $\Delta 5$ -elongase from *Ostreococcus tauri* (SEQ ID NO: 64) and the open reading frame of the $\Delta 4$ -desaturase from *Thraustochytrium* sp. (SEQ ID NO: 77) was cloned into pUC19-35S. The resulting plasmids
25 pUC-E5 and pUC-D4 were then transferred via Sall/XhoI in accordance with the above statements into the vector pGPTV-D6D5E6(Tp) $\omega 3$ Pi. The resulting vector pGPTV-D6D5E6(Tp) $\omega 3$ PiE5D4 was employed for the plant transformation.

All the binary vectors were transformed into *E. coli* DH5 α cells (Invitrogen) in accordance with the manufacturer's instructions. Positive clones were identified by PCR, and plasmid
30 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 cm²) 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 cm²) 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.

10 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.

30 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 $\Delta 5$ -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% CaCl₂ (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: $sn-1 + sn-3 = (TAG \times 3 - MAG)/2$.

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 N₂ and resuspended in 0.5 ml of borate buffer (0.5M, pH 7.5, containing 0.4 mM CaCl₂). 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 <i>Ostreococcus tauri</i> 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 <i>Orychophragmus violaceus</i>											
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 <i>Arabidopsis thaliana</i>											
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-7	18:2n-6	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	22:6n-3
wt	25.12	3.83	5.06		18.53		44.72							
sn-2	1.42	0.76	8.79		27.62		62.03							
sn-1+3	36.97	4.17	4.19		13.98		38.07							
Transgene	22.63	3.12	3.46	0.77	2.35	9.51	6.37	13.93	0.74	0.83	3.87	24.96	2.22	4.15
sn-2	1.62	0.64	8.33	1.61	5.16	16.21	19.88	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	0.98	6.18	4.11	9.63	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	10.66	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	264	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	0.03	0.17	0.87	3.85
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

DGCG	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	63.74					
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	54.65	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	55.05	0.27	0.00	0.00	0.35	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	γ 18:3	α 18:3	18:4	20:0
BJ223_PUFA104_MKP71_581A	4.4	3.0	22.5	16.9	27.0	4.9	3.2	0.6
BJ223_PUFA104_MKP71_581A	4.7	3.9	17.9	10.6	29.5	4.2	4.0	0.9
BJ223_PUFA104_MKP71_581A	4.4	3.0	18.9	13.8	30.5	4.1	3.2	0.7
BJ223_PUFA104_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 (ETE4) (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)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response_NonFinal.pdf	78880 e08bcbb5537e3923d53d8e03c88fe049249ec20	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:

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2	Specification	Substitute_Specification.pdf	714422	no	86
			16809a6adef0815e19d6803d27babf9641188e2b		

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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.

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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	<input type="checkbox"/> To be Mailed
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ENTITY: LARGE SMALL MICRO

APPLICATION AS FILED – PART I

FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)
<input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
<input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A	
<input type="checkbox"/> 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 \$ =	
<input type="checkbox"/> 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).			
<input type="checkbox"/> 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

	(Column 1)	(Column 2)	(Column 3)	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT	10/23/2017	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		
	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
	<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))					
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

	(Column 1)	(Column 2)	(Column 3)	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$ =
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$ =
	<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))					
<input type="checkbox"/> 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.

LIE
EVELYN NIMMONS

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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				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	
Sheet	1	of	2	Attorney Docket Number	074017-0013-01-US	

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
	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	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
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)				
	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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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				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	
Sheet	2	of	2	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.	
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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.	
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	CM	KANG, Z. B., et al., "Adenoviral Gene Transfer of <i>Caenorhabditis elegans</i> n-3 Fatty Acid Desaturase Optimizes Fatty Acid Composition in Mammalian Cells", PNAS, 2001, Vol. 98, No. 7, pp. 4050-4054.	

Examiner Signature		Date Considered	
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¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES
PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro



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25. Juli 2002 (25.07.2002)

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- (51) Internationale Patentklassifikation⁷: C12N 15/82, 15/53, 15/11, 9/02, C12P 7/62, 7/64, C12Q 1/68, C07K 16/40, G01N 33/573, C11B 1/00
- (71) Anmelder (für alle Bestimmungsstaaten mit Ausnahme von US): BASF PLANT SCIENCE GMBH [DE/DE]; 67056 Ludwigshafen (DE).
- (21) Internationales Aktenzeichen: PCT/EP02/00462
- (72) Erfinder; und
- (22) Internationales Anmeldedatum: 18. Januar 2002 (18.01.2002)
- (75) Erfinder/Anmelder (nur für US): LERCHL, Jens [DE/SE]; Onsjövägen 17, S-26831 Svalöv (SE). RENZ, Andreas [DE/DE]; Heinrich-von-Kleist-Str. 6, 67117 Limburgerhof (DE). HEINZ, Ernst [DE/DE]; Püttkampsweg 13, 22609 Hamburg (DE). DOMERGUE, Frederic [FR/DE]; Bahrenfelder Steindamm 98, 22761 Hamburg (DE). ZÄHRINGER, Ulrich [DE/DE]; Theodor-Storm-Strasse 34a, 22926 Ahrensburg (DE).
- (25) Einreichungssprache: Deutsch
- (26) Veröffentlichungssprache: Deutsch
- (30) Angaben zur Priorität: 101 02 337.5 19. Januar 2001 (19.01.2001) DE

[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 FETTSÄUREN, 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 C₁₈, C₂₀, or C₂₂ 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 höchtem 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 Organismus bevorzugt einer transgenen Pflanze oder eines transgenen Mikroorganismus mit erhöhtem Gehalt an Fettsäuren, Ölen oder Lipiden mit ungesättigten C₁₈-, C₂₀-, oder C₂₂-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 Nukleinsäuresequenzen. 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]



WO 02/057465 A2



(74) **Anwalt:** GOLDSCHIED, Bettina; BASF Aktiengesellschaft, 67056 Ludwigshafen (DE).

(81) **Bestimmungsstaaten (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) **Bestimmungsstaaten (regional):** ARIPO-Patent (GI, GM, KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZM, ZW),

eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI-Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Verfahren zur Herstellung mehrfach ungesättigter Fettsäuren, neue Biosynthesegene sowie neue pflanzliche Expressionskonstrukte

5 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 Organismus bevorzugt einer transgenen Pflanze oder eines transgenen Mikroorganismus mit erhöhtem Gehalt an Fettsäuren, Ölen oder Lipiden mit ungesättigten C₁₈-, C₂₀- oder C₂₂-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 Nukleinsäuresequenzen, 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

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"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 tricorutum* oder *Cryptocodinium*-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 Mutantpflanzen 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 C₁₈-, C₂₀-Fettsäuren und C₂₂-Fettsäuren und solchen mit längeren Kohlenstoffketten.

3

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, 10 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: 15 649-659 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 20 (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 25 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 Fettsäurespektrums zu ungesättigten Fettsäuren hin konnte gezeigt werden, gleichzeitig zeigte sich aber, 30 dass die Syntheseleistung der transgenen Pflanzen stark nachließ, das heißt gegenüber den Ausgangspflanzen konnten nur geringere Mengen an Ölen isoliert werden.

35 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.

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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 45 biotechnologischen Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren liefert die vorgenannten Fettsäuren in wirtschaftlich nutzbaren Mengen.

4

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:

10

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,

15 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,

20 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,

25

in einen Fettsäureester produzierenden Organismus einbringt, anzieht und die dem Organismus enthaltenden Fettsäureester isoliert.

30

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

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codieren.

Im erfindungsgemäßen Verfahren werden vorteilhaft Fettsäureester mit mehrfach ungesättigten C₁₈-, C₂₀- und/oder C₂₂-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).

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45 Die Fettsäureester mit mehrfach ungesättigten C₁₈-, C₂₀- und/oder C₂₂-Fettsäuremolekülen können aus den Organismen, die für die Herstellung der Fettsäureester verwendet wurden, in Form eines Öls

5

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 prinzipiell alle prokaryontischen oder eukaryontischen Organismen wie prokaryontische oder eukaryontische 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 *Cryptocodium*, *Thraustochytrium*, *Phaeodactylum* und *Mortierella*, *Entomophthora*, *Mucor*, *Cryptocodium* 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 (Ölpalme, 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

6

- 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
- 5 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
- 10 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.
- 15
- 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. Transgen bedeutet
- 20 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 dass die Regulationssequenzen, der natürlichen Sequenzen verändert wurden. Bevorzugt ist unter transgen die Expression
- 25 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.
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- 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
- 35 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 Fettsäure-
- 40 molekülen 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 Zusammen-
- 45 setzungen 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

5 Desaturaseaktivität allein oder in Kombination oder die Desaturasenukleinsäureexpression moduliert, so dass eine zell-assoziierte Aktivität relativ zu der gleichen Aktivität in Abwesenheit der Substanz verändert wird. Bei einer bevorzugten Ausführungsform wird/werden ein oder zwei Stoffwechselweg(e)

10 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 Desaturase-

15 aktivität 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

20 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.

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Ein weiterer Aspekt der Erfindung betrifft Verfahren zur Modulation der Ausbeuten einer gewünschten Verbindung aus einer Zelle, umfassend das Einbringen eines Wildtyp- oder Mutanten-

30 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

35 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

40 verbunden ist.

Bei einer bevorzugten Ausführungsform sind die Ausbeuten modifiziert. Bei einer weiteren bevorzugten Ausführungsform wird die gewünschte Chemikalie vermehrt, wobei unerwünschte

45 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).

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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 *Cryptocodium*, *Thraustochytrium*, *Phaeodactylum* und *Mortierella*, *Entomophthora*, *Mucor*, *Cryptocodium* 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 tricorutum*, beschrieben. Dadurch können die oben genannten Nukleinsäuremoleküle im erfindungsgemäßen Verfahren, indem die Organismen 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 Mikroorganismen, 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,

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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

5 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 Triacylglycerolfraktion erhalten werden. Weiterhin wurden

10 Δ -4-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.

15

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

20 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

25 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

30 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

35 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

40 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

45 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

5 höheren Desaturierungsgrad, wie Dihomo- γ -Linolensäure, Docosadiensäure, Arachidonsäure, ω 6-Eicosatriendihomo- γ -linolensäure, Eicosapentaensäure, ω 3-Eicosatriensäure, ω 3-Eicosatetraensäure, Docosapentaensäure oder Docosahexaensäure. Substrate der erfindungsgemäßen Enzymaktivität sind zum Beispiel Taxolsäure;

10 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,

15 Eicosapentaensäure. Besonders bevorzugt als Produkte des erfindungsgemäßen Verfahrens sind Arachidonsäure, Docosapentaensäure, Eicosapentaensäure. Die C₁₈-Fettsäuren mit mindestens zwei Doppelbindungen in der Fettsäure können durch die erfindungsgemäße enzymatische Aktivität in Form der freien Fettsäure oder

20 in Form der Ester, wie Phospholipide, Glycolipide, Sphingolipide, Phosphoglyceride, Monoacylglyceride, Diacylglyceride oder Triacylglyceride, verlängert werden.

Für die menschliche Ernährung ist konjugierte Linolsäure "CLA"

25 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

30 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,

35 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

40 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

45 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

12

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.

- 5 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.
- 10 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.
- 15 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 ent-
- 20 sprechenden 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
- 25 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
- 30 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,
- 35 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,
- 40 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
- 45 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
5 weiterhin zu einem Desaturaseprotein mit geänderten Aktivitäten
führen, welche die Produktion einer oder mehrerer gewünschter
Feinchemikalien direkt oder indirekt beeinflussen. Beispiels-
weise kann die Anzahl oder Aktivität der/des erfindungsgemäßen
Desaturasegens(e) gesteigert werden, so dass die normalen Stoff-
10 wechselabfälle oder -nebenprodukte der Zelle (deren Menge mög-
licherweise aufgrund der Überproduktion der gewünschten Fein-
chemikalie erhöht ist) effizient exportiert werden, bevor sie
andere Moleküle oder Prozesse innerhalb der Zelle (welche die
Lebensfähigkeit der Zelle senken würden) zerstören oder die Bio-
15 synthesewege der Feinchemikalie stören würden (wodurch die Aus-
beute, 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,
20 wie die allosterische Regulation, stören, beispielsweise könnte
sie durch Steigerung der Aktivität oder Anzahl anderer strom-
abwärts folgender Enzyme oder Entgiftungsenzyme des PUFA-Wegs
die Allokation der PUFA in die Triacylglycerin-Fraktion steigern,
man könnte die Lebensfähigkeit von Saatzellen erhöhen, was
25 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 ein-
30 schneidende 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 erheb-
35 lich 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 Merk-
40 male, wie Toleranz gegenüber abiotischen und biotischen Stress-
situationen, beeinflussen.

Biotische und abiotische Stresstoleranz ist ein allgemeines
Merkmal, das man an ein breites Spektrum von Pflanzen, wie
45 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
5 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,
10 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
15 Teile davon codieren, oder Nukleinsäurefragmente, die sich als Primer oder Hybridisierungs sonden 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 dar-
20 gestellten Nukleotidsequenzen oder die kodierende Region oder ein Komplement einer dieser Nukleotidsequenzen. Bei anderen besonders bevorzugten Ausführungsformen umfasst das erfindungsgemäße iso- lierte Nukleinsäuremolekül eine Nukleotidsequenz, die an eine Nukleotidsequenz, wie in der Sequenz SEQ ID NO: 1, 3, 5 oder 11
25 dargestellt, oder einen Teil davon hybridisiert oder zu minde- stens 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
30 kodiert das isolierte Nukleinsäuremolekül eine der in der Sequenz SEQ ID NO: 2, 4, 6 oder 12 dargestellten Aminosäuresequenzen. Das bevorzugte erfindungsgemäße Desaturasegen besitzt vorzugs- weise auch mindestens eine der hier beschriebenen Desaturase- aktivitäten.

35 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
40 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
45 am Transport von Molekülen über diese Membranen teilzunehmen. Bei einer Ausführungsform ist das von dem Nukleinsäuremolekül kodierte Protein zu mindestens etwa 50 %, vorzugsweise mindestens

15

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.

10

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 C₁₈- oder C₂₀₋₂₂-Kohlenstoffketten mit Doppelbindungen an mindestens zwei Stellen gemeint ist.

20 Bei einer anderen bevorzugten Ausführungsform rührt das isolierte Nukleinsäuremolekül von *Phaeodactylum tricornerutum* 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.

45 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

5 natürlich vorkommende Phaeodactylum-Desaturase oder einen biologisch aktiven Teil davon.

Eine weitere Ausführungsform der Erfindung sind Expressionskassetten, die die Expression der erfindungsgemäßen Nukleinsäuren

10 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.

15 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

20 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

25 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

30 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

35 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

40 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

45 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
5 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
10 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 bei-
15 spielsweise 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
20 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.
25 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 Speicher-
geweben, wie Samenhüllen, Knollen, Epidermis- und Samenzellen,
30 Endosperm oder Embryogewebe 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 Lein-
35 pflanze, 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 endo-
40 genes 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
45 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
5 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
10 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
15 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
20 teilzunehmen, beibehält.

Die Erfindung stellt auch eine isolierte Präparation einer Desaturase in Form eines Rohextraktes oder als reines Protein bereit.

25 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
30 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
35 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.
40 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
45 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

19

Multiexpressionskassetten definiert durch SEQ ID NO: 13, 14, 15, 16 und 17 eingefügt zur Transformation von Pflanzen und Expression in Pflanzen geeignet sind.

5 Eingehende Beschreibung der Erfindung

Ein erfindungsgemäßer Gegenstand ist/sind isolierte Nukleinsäuresequenz(en), die für ein Polypeptid mit Desaturaseaktivität codiert, ausgewählt aus der Gruppe:

10

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,

15 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,

20 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
25 Polypeptide wesentlich reduziert ist.

Weiterhin betrifft die Erfindung eine Aminosäuresequenz, die durch die oben genannte(n) Nukleinsäuresequenz(en) codiert werden
30 (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.

35 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
40 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,
45 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

5 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 Ver-

10 bindungen 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

15 Feinchemikalien beeinflussen kann). Aspekte der Erfindung sind nachstehend weiter erläutert.

I. Feinchemikalien und PUFAs

20 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 um-

25 fassen 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),

30 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

35 Scientific and Technological Associations in Malaysia and the Society for Free Radical Research - Asien, abgehalten am 1.-3. Sept. 1994 in Penang, Malaysia, AOCs Press (1995)), Enzyme und sämtliche anderen von Gutcho (1983) in Chemicals by Fermentation, Noyes Data Corporation, ISBN: 0818805086, und darin angegebenen

40 Literaturstellen beschriebenen Chemikalien. Der Stoffwechsel und die Verwendungen bestimmter Feinchemikalien sind nachstehend weiter erläutert.

Die Kombination verschiedener Vorläufermoleküle und Biosynthese-

45 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,
 5 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.

10 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
 15 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,
 20 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
 25 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
 30 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
 35 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
 40 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
 45 Literaturstellen; Lengeler et al. (Hrsgb.) (1999) *Biology of Prokaryotes*. 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, 5 Linol- und Linolensäure. Diese C₁₈-Kohlenstoff-Fettsäuren müssen auf C₂₀ und C₂₂ 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- 10 aktivität aufweisen, können Arachidonsäure, Eicosapentaensäure und Docosahexaensäure sowie verschiedene andere langkettige PUFAs erhalten, extrahiert und für verschiedene Zwecke bei Nahrungs- mittel-, Futter-, Kosmetik- oder pharmazeutischen Anwendungen verwendet werden.
- 15 Zur Herstellung langkettiger PUFAs müssen, wie oben erwähnt, die mehrfach ungesättigten C₁₈- bzw C₂₀-Fettsäuren mehrfach desaturiert werden. Die erfindungsgemäßen Nukleinsäuresequenzen kodieren erste funktionell aktive Desaturasen aus *Phyeodactylum* 20 *tricornutum*, einem Mikroorganismus, der PUFAs in der Triacyl- glycerolfraktion enthält. Mit den erfindungsgemäßen Desaturasen können Doppelbindungen in die Δ-5-, Δ-6- oder Δ-12-Position ein- geführt werden. Die Aktivitäten der erfindungsgemäßen Desaturasen führt vorzugsweise zu C₁₈- + C₂₀-Fettsäuren mit mindestens zwei, 25 drei, vier oder fünf Doppelbindungen im Fettsäuremolekül, vor- zugsweise zu C₂₀-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 mög- 30 lichen weiteren Desaturierung und Elongation zu bevorzugten PUFAs mit höherem Desaturierungsgrad, einschließlich einer weiteren Elongation von C₂₀ zu C₂₂-Fettsäuren, zu Fettsäuren wie Linolsäure, Docosadiensäure, dihomο-γ-linolensäure, Arachidonsäure, ω6-Eicosa- triendihomο-γ-linolensäure, Eicosapentaensäure, ω3-Eicosatrien- 35 säure, ω3-Eicosatetraensäure, Docosapentaensäure oder Docosa- hexaensäure. Substrate dieser erfindungsgemäßen Enzymaktivität sind zum Beispiel Taxolsäure, 6,9-Octadecadiensäure, Ölsäure, Linolsäure, γ-Linolensäure, Pinolensäure, α-Linolensäure, Ara- chidonsäure, Eicosapentaensäure oder Stearidonsäure. Bevorzugte 40 Substrate sind Linolsäure, γ-Linolensäure und/oder α-Linolensäure, dihomο-γ-linolensäure bzw. Arachidonsäure, Eicosatetraensäure oder Eicosapentaensäure. Die C₁₈-oder C₂₀-Fettsäuren mit min- destens zwei Doppelbindungen in der Fettsäure können durch die erfindungsgemäße Enzymaktivität in Form der freien Fettsäure oder 45 in Form der Ester, wie Phospholipide, Glykolipide, Sphingolipide, Phosphoglyceride, Monoacylglyceride, Diacylglyceride, Triacylgly- ceride 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 5 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 10 von fetthaltigen Verbindungen, die Betaoxidation, Fettsäuremodifikation und Cofaktoren, Triacylglycerin-Speicherung und -Assemblierung einschließlich der Literaturstellen darin siehe in den folgenden Artikeln: Kinney, 1997, *Genetic Engineering*, Hrsgb.: JK Setlow, 19:149-166; Ohlrogge und Browse, 1995, *Plant* 15 *Cell* 7:957-970; Shanklin und Cahoon, 1998, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:611-641; Voelker, 1996, *Genetic Engineering*, 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.* 20 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.

25 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 30 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, 35 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 40 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 45 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.
- 5 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
- 10 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.

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II. Elemente und Verfahren der Erfindung

- Die vorliegende Erfindung beruht unter anderem auf der Entdeckung neuer Moleküle, die hier als Desaturasenukleinsäure- und
- 20 -proteinmoleküle bezeichnet werden, welche eine Wirkung auf die Produktion von Zellmembranen und Lipiden *Phaeodactylum tri-*cornutum ausüben und beispielsweise die Bewegung von Molekülen über diese Membranen beeinflussen. Bei einer Ausführungsform nehmen die Desaturasemoleküle am Stoffwechsel von zum Aufbau von
- 25 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 Membran-
- 30 komponenten 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
- 35 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
- 40 Produktion einer gewünschten Feinchemikalie durch Mikroorganismen und Pflanzen moduliert.

- Der Begriff "Desaturase" oder "Desaturasepolypeptid" umfasst Proteine, die an der Desaturierung von Fettsäuren teilnehmen.
- 45 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, 5 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. 10 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 Auf-
richtung einer bestimmten Durchsatzrate einer Feinchemikalie benötigt). Der Begriff Ausbeute oder Produkt/Kohlenstoff-
15 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
20 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 Ver-
25 bindung, 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
30 (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
35 Verbindung (z.B. der Stoffwechsel einer Fettsäure) umfasst dann die Gesamtheit der Biosynthese-, Modifikations- und Abbauege dieser Verbindung in der Zelle, die diese Verbindung betreffen.

Bei einer anderen Ausführungsform können die erfindungsgemäßen
40 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
45 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
5 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 Aktivi-
10 tät 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.

15

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. Beispiels-
20 weise 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
25 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
30 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
35 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 Zell-
40 membran 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
45 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 5 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) 10 oder die Kältetoleranz, wie offenbart in WO 95/18222, beeinflussen.

Die erfindungsgemäßen isolierten Nukleinsäuresequenzen sind im Genom eines *Phaeodactylum tricornutum* UTEX646-Stammes enthalten, 15 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 20 SEQ ID NO: 1 bis 6 sowie 11 und 12 gezeigt. Es wurden Computeralysen 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 25 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 Klonen 30 PT001078032R dargestellt in SEQ ID NO: 1. Den Klonen wurden Genamen 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 35 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 wer- 40 den. 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 wei- 45 tere Nukleinsäuresequenz, die für eine Δ -12-Desaturase codiert,

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ist Tabelle 1 zu entnehmen. Sie trägt die Klon-Nummer PT001072031R.

Tabelle 1

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	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
10	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

- 15 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.
- 30 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 C₁₈-bzw C₂₀₋₂₂-PUFAs benötigten Aktivitäten beitragen, so dass C₁₈, C₂₀-, C₂₂- oder C₂₄-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 Agrobacterium eingesetzt werden.

Verschiedene Aspekte der Erfindung sind eingehender in den folgenden Unterabschnitten beschrieben.

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A. Isolierte Nukleinsäuremoleküle

Eine Ausführungsform der Erfindung sind isolierte Nukleinsäuren, die von PUFA produzierenden Mikroorganismen stammen und für Poly-
 5 peptide kodieren, die C₁₈-oder C₂₀₋₂₂-Fettsäuren mit mindestens einer, zwei, drei oder vier Doppelbindungen in der Fettsäure desaturieren.

Eine weitere erfindungsgemäße Ausführungsform sind isolierte
 10 Nukleinsäuren, umfassend Nukleotidsequenzen, die für Polypeptide kodieren, die C₁₈-bzw C₂₀-Fettsäuren mit mindestens ein, zwei, drei oder vier Doppelbindungen in der Fettsäure desaturieren und sind aus der Gruppe, bestehend aus

- 15 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
 20 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
 25 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
 30 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*
 35 *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
 40 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,
 45 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.
- 5 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,
- 10 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
- 15 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-
- 20 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.
- 25 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 Sequenz-
- 30 information 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 tricorutum* cDNA aus einer *Phaeodactylum tricorutum*-Bank iso-
- 35 liert 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,
- 40 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
- 45 Regionen um Motive aus Beispiel 5a erstellt werden oder Modifikationen ebensolcher in einzelnen definierten Aminosäuren, verwendet werden (z.B. kann ein Nukleinsäuremolekül, umfassend die

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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

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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 5 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 10 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 15 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 20 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 25 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 30 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 35 Modifikation ihrer Sequenz erhöht ist oder dass sie vollständig durch aktivere Promotoren, sogar aus heterologen Organismen, ersetzt werden.

Überdies kann das erfindungsgemäße Nukleinsäuremolekül nur 40 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 45 von *Phaeodactylum tricorutum* ermittelten Nukleotidsequenzen ermöglichen die Erzeugung von Sonden und Primern, die zur Identifizierung und/oder Klonierung von Desaturase-Homologen in anderen

33

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

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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
- 10 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
- 15 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
- 20 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 bio-

25 logisch 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

30 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 Trans-

35 port 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.

- 40 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)
- 45 und Bestimmen der Aktivität des kodierten Teils der Desaturase oder des Peptids herstellen.

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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

5 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.

10 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

15 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 tricorutum*-Population) existieren können. Diese genetischen Polymorphismen im Desaturase-Gen können zwischen Individuen innerhalb einer Population aufgrund von natürlicher Variation existieren. Wie hier verwendet, bedeuten

20 die Begriffe "Gen" und "rekombinantes Gen" Nukleinsäuremoleküle mit einem offenen Leserahmen, der eine Desaturase, vorzugsweise eine *Phaeodactylum tricorutum* -Desaturase, kodiert. Diese natürlichen Varianten bewirken üblicherweise eine Varianz von 1 bis 5 % in der Nukleotidsequenz des Desaturase-Gens. Sämtliche

30 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.

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Nukleinsäuremoleküle, die den natürlichen Varianten entsprechen, und nicht-*Phaeodactylum tricorutum*-Homologen, -Derivate und -Analoge der *Phaeodactylum tricorutum*-cDNA können auf der Grundlage ihrer Homologie zu der hier offenbarten *Phaeodactylum*

40 *tricorutum*-Desaturase-Nukleinsäure unter Verwendung der *Phaeodactylum tricorutum*-cDNA oder eines Teils davon als Hybridisierungssonde gemäß Standard-Hybridisierungstechniken unter stringenten Hybridisierungsbedingungen isoliert werden. Bei einer anderen Ausführungsform ist ein erfindungsgemäßes isoliertes

45 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.

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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/sodium citrate = SSC) bei etwa 45°C, gefolgt von einem oder mehreren Waschschrritten 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 oben genannten 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-

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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 5 kodiert die Nukleinsäure eine natürliche vorkommendes Phaeodactylum tricornutum-Desaturase.

Zusätzlich zu natürlich vorkommenden Varianten der Desaturase-sequenz, die in der Population existieren können, erkennt der 10 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 15 lassen sich Nukleotidsusbtitutionen, 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 20 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 25 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 30 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 35 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 Zell- 40 membranen 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 min- 45 destens 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.

- 5 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
- 10 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.
- 15 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
- 20 (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
- 25 identischen Positionen/Gesamtanzahl der Positionen x 100). Die Begriffe Homologie und Identität sind damit als Synonym anzusehen.

- Ein isoliertes Nukleinsäuremolekül, das eine Desaturase kodiert,
- 30 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
- 35 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
- 40 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
- 45 definiert worden. Diese Familien umfassen Aminosäuren mit basischen Seitenketten (z.B. Lysin, Arginin, Histidin), sauren Seitenketten (z.B. Asparaginsäure, Glutaminsäure), ungeladenen

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- 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. 5 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 10 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, 15 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.
- 20
- 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 "Anti- 25 sense"-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 Wasserstoff- 30 brückenbindungen 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 35 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).
- 40 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 45 nicht in Aminosäuren translatiert werden (d.h. die man auch als 5'- und 3'-untranslatierte Bereiche bezeichnet).

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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-Basen-

5 paarung 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

10 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

15 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

20 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 Nukleo-

25 tide, 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-(Carboxyhydroxymethyl)uracil, 5-Carboxymethylaminomethyl-2-thiouridin, 5-Carboxymethylaminomethyluracil, Dihydrouracil,

30 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'-Methoxycarboxymethyl-

35 uracil, 5-Methoxyuracil, 2-Methylthio-N6-isopentyladenin, Uracil-5-oxycyessigsäure (v), Wybutoxosin, Desaturaseudouracil, Queosin, 2-Thiocytosin, 5-Methyl-2-thiouracil, 2-Thiouracil, 4-Thiouracil, 5-Methyluracil, Uracil-5-oxycyessigsäuremethylester, Uracil-5-oxycyessigsäure (v), 5-Methyl-2-thiouracil, 3-(3-Amino-3-N-2-

40 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

45 zu einer Zielnukleinsäure von Interesse in Antisense-Richtung orientiert, was im nachstehenden Unterabschnitt weiter beschrieben ist).

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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-Duplexes 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 -anomerer Nukleinsäuremolekül. Ein α -anomerer 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 gelehrt Verfahren zu isolierenden heterologen

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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
5 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
10 261:1411-1418.

Alternativ lässt sich die Desaturase-Gen-Expression hemmen, indem Nukleotidsequenzen, die komplementär zum regulatorischen Bereich einer Desaturase-Nukleotidsequenz (z.B. einem Desaturase-
15 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.
20 (1992) Bioassays 14(12):807-815.

B. Genkonstrukt (= Nukleinsäurekonstrukt, -fragment oder Expressionskassette)

25 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
30 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
35 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
40 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
45 Expression der Gene erhöht wurde. Das Genkonstrukt kann aber auch einfacher aufgebaut sein, das heißt es wurden keine zusätzlichen Regulationssignale vor die Nukleinsäuresequenz oder

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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

5 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

10 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

15 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

20 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 bei-

25 spielsweise 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.

30 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 Doppel-

35 bindungen 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

40 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

45 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.

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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

5 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

10 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

15 einer oder mehreren Kopien vorliegen. Sie können in einem Genkonstrukt oder mehreren Genkonstrukten vorliegen. Dieses Genkonstrukt oder die Genkonstrukte können zusammen im Wirtorganismus exprimiert werden. Dabei kann das Genkonstrukt oder die Genkonstrukte in einem oder mehreren Vektoren inseriert sein

20 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

25 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

30 Promotoren *amy* und *SPO2*, in den Hefe- oder Pilzpromotoren *ADC1*, *MF α* , *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

35 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 (Abzisinäure-induzierbar) oder WO 93/21334 (Ethanol- oder Cyclohexenol-induzierbar) beschriebenen Promotoren. Weitere geeignete Pflanzenpromotoren sind

40 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

45 beschriebene nodienspezifische Promotor. Besonders vorteilhafte Promotoren sind Promotoren, welche die Expression in Geweben ermöglichen, die an der Fettsäurebiosynthese beteiligt sind. Ganz

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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

5 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

10 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-

15 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

20 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

25 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

30 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 Nukleinsäure-

35 konstrukten 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),

40 Acetyl-Coenzym A-Carboxylase(n), Acyl-Coenzym, A-Oxidase(n), Fettsäure-Desaturase(n), Fettsäure-Acetylasen, Lipoxygenasen, Triacylglycerol-Lipasen, Allenoxid-Synthasen, Hydroperoxid-Lyasen oder Fettsäure-Elongase(n) oder deren Kombinationen verwendet.

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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 5 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 10 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 ein- 15 gebrachten 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 20 durch Verbesserung der mRNA-Stabilität zu verstärken.

C. Rekombinante Expressionsvektoren und Wirtszellen

Ein weiterer Aspekt der Erfindung betrifft Vektoren, vorzugsweise 25 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 30 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- 35 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 40 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 45 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

5 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,

10 Transposons, IS-Elemente, Phasmide, Phagemide, Cosmide, lineare oder zirkuläre DNA, umfassen.

Die erfindungsgemäßen rekombinanten Expressionsvektoren umfassen eine erfindungsgemäße Nukleinsäure oder ein erfindungsgemäßes

15 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

20 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

25 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

30 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

35 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

40 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

45 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
5 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-Expressions-
vektoren), Hefe- und anderen Pilzzellen (siehe Romanos, M.A.,
10 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,
15 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: Holo-
20 trichia, Peritrichia, Spirotrichia, Suctoria, Tetrahymena, Para-
mecium, Colpidium, Glaucoma, Platyophrya, Potomacus, Desaturase-
udocohnilembus, Euplotes, Engelmaniella und Stylonychia, ins-
besondere der Gattung Stylonychia lemnae, mit Vektoren nach einem
Transformationsverfahren, wie beschrieben in WO 98/01572, sowie
25 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,
30 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
35 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
40 T7-Polymerase, in vitro transkribiert und translatiert werden.

Die Expression von Proteinen in Prokaryoten erfolgt meist mit
Vektoren, die konstitutive oder induzierbare Promotoren ent-
halten, welche die Expression von Fusions- oder nicht-Fusions-
45 proteinen steuern. Fusionsvektoren fügen eine Reihe von Amino-
säuren an ein darin kodiertes Protein an, gewöhnlich am Amino-
terminus 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
- 5** 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
- 10** Proteins von der Fusionseinheit nach der Reinigung des Fusionsproteins möglich ist. Diese Enzyme und ihre entsprechenden Erkennungssequenzen umfassen Faktor Xa, Thrombin und Entero-kinase.
- 15** 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
- 20** 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
- 25** 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.
- 30** 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
- 35** 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
- 40** 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

45 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, λ gt11 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

5 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

10 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

15 Veränderung der erfindungsgemäßen Nukleinsäuresequenzen erfolgt durch Standard-DNA-Synthesetechniken.

Bei einer weiteren Ausführungsform ist der Desaturase-Expressionsvektor ein Hefe-Expressionsvektor. Beispiele für

20 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 Kon-

25 struktion 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, F.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: *Applied Molecular Genetics of fungi*,

30 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, YE_p6, YE_p13 oder pEMBLYe23.

35 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) ver-

40 fügbar 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

45 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 erfindungs-
 5 gemäße 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)
 10 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, Cytomegalie-
 virus und Simian Virus 40. Weitere geeignete Expressionssysteme
 15 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.

20 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).
 25 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),
 30 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), pankreas-
 35 spezifische Promotoren (Edlund et al., (1985) Science
 230:912-916) und milchdrüsenspezifische Promotoren (z.B. Milch-
 serum-Promotor; US-Patent Nr. 4,873,316 und Europäische Patent-
 anmeldung-Veröffentlichung Nr. 264,166). Auch entwicklungs-
 regulierte Promotoren sind umfasst, z.B. die hox-Promotoren
 40 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
 45 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
5 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
10 Utilization, Hrsgb.: Kung und R. Wu, Academic Press, 1993, S. 15-38.

Eine Pflanzen-Expressionskassette enthält vorzugsweise Regulationssequenzen, welche die Genexpression in Pflanzen-
15 zellen 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 Octopinsynthese
20 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.

25 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 Tabakmosaik-
30 virus, 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
35 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
40 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
45 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, 5 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).

- 10 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 15 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.

- 20 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).

- 25 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 30 (US 5,608,152), der USP-Promotor aus *Vicia faba* (Bäumlein 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; 35 Bäumlein 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) 40 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).

45

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
5 mehrerer einzelner Expressionskonstrukte erfolgen oder durch Kombination mehrerer Expressionskassetten auf einem Konstrukt. Auch können mehrere Vektoren mit jeweils mehreren Expressionskassetten transformiert und auf die Wirtszelle übertragen werden.

10

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
15 Promotoren, wie der virale RNA-Polymerase-Promotor, sind beschrieben in WO 95/15783 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
25 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
30 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
35 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.,
40 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
45 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
5 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
10 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
15 Herkunftstorganismus verändert oder in diesem wurden die Nukleinsäuresequenzen und/oder die Regulationssequenzen mutiert oder die erfindungsgemäßen Nukleinsäuresequenzen wurden in einen anderen Organismus als den Herkunftstorganismus verbracht oder deren Regulationssequenzen. Auch Kombinationen dieser Veränderungen sind möglich. Unter natürlicher Umgebung ist die Lage einer
20 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,
25 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.

30 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
35 Vielzahl von im Stand der Technik bekannten Verfahren zum Einbringen fremder Nukleinsäure (z.B. DNA) in eine Wirtszelle, einschließlich Calciumphosphat- oder Calciumchlorid-Coprecipitation, DEAE-Dextran-vermittelte Transfektion, Lipofektion, natürliche Kompetenz, chemisch vermittelter Transfer, Elektro-
40 poration 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
45 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,
5 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 Integrierten wird gewöhnlich ein Gen, das einen selektier-
baren Marker (z.B. Resistenz gegen Antibiotika) kodiert, zusammen
10 mit dem Gen von Interesse in die Wirtszellen eingebracht. Bevor-
zugte selektierbare Marker umfassen solche, welche Resistenz
gegen Medikamente, wie G418, Hygromycin und Methotrexat, ver-
leihen, oder in Pflanzen solche, welche Resistenz gegen ein
Herbizid, wie Glyphosphat oder Glufosinat, verleihen. Weitere ge-
15 eignete Marker sind beispielsweise Marker, welche Gene kodieren,
die an Biosynthesewegen von zum Beispiel Zuckern oder Aminosäuren
beteiligt sind, wie β -Galactosidase, *ura3* oder *ilv2*. Marker,
welche Gene, wie Luziferase, *gfp* oder andere Fluoreszenzgene
kodieren, sind ebenfalls geeignet. Diese Marker lassen sich in
20 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 Wirts-
zelle auf dem gleichen Vektor eingebracht werden, wie derjenige,
25 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, wohin-
30 gegen 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
35 Substitution eingebracht worden ist, um dadurch das Desaturasegen
zu verändern, z.B. funktionell zu disruptieren. Dieses Desatura-
segen ist vorzugsweise ein *Phaeodactylum tricornutum* Desaturase-
gen, es kann jedoch ein Homologon oder Analogon aus anderen
Organismen, sogar aus einer Säuger-, Pilz- oder Insektenquelle
40 verwendet werden. Bei einer bevorzugten Ausführungsform ist der
Vektor so gestaltet, dass das endogene Desaturasegen bei homo-
loger Rekombination funktionell disruptiert wird (d.h. nicht
länger ein funktionelles Protein kodiert, auch als Knock-out-
Vektor bezeichnet). Alternativ kann der Vektor so gestaltet
45 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, 1999, 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) 5 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 erfindungs- 10 gemäßen 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 vor- 15 teilhafte 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, 20 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 25 (Ölplume, Kokosnuß) sowie ausdauernde Gräser und Futterfeldfrüchte. Besonders bevorzugte erfindungsgemäße Pflanzen sind Ölfruchtpflanzen, wie Soja, Erdnuss, Raps, Canola, Lein, Nachtkerze, Sonnenblume, Safflor, Bäume (Ölpalme, Kokosnuß).

30 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 35 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 zellu- 40 lären 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 45 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
- 5 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
- 10 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
- 15 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
- 20 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.
- 25 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.
- 30 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
- 35 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
- 40 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
- 45 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 5
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
10 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 Stoff-
15 wechsel 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 C₁₈, C₂₀ oder C₂₂ einführt.

20

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
25 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
30 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
35 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.

40 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
45 weniger Aminosäuren als die Vollängen-Desaturase oder das Vollängenprotein aufweisen, das zu einer Desaturase homolog ist, und zumindest eine Aktivität einer Desaturase aufweisen.

61

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.

5 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
10 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
15 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-
20 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
25 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
30 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
35 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 von der Desaturase unterscheidet und aus dem gleichen oder einem
40 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
45 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
5 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.

10

Ein erfindungsgemäßes chimäres Desaturase-Protein oder Desaturase-Fusionsprotein wird durch Standard-DNA-Rekombinationstechniken hergestellt. Zum Beispiel werden DNA-Fragmente, die unterschiedliche Polypeptidsequenzen kodieren, gemäß herkömm-
15 licher Techniken im Leseraster aneinander ligiert, indem beispielsweise glatte oder überhängende Enden zur Ligation, Restriktionsenzymsspaltung zur Bereitstellung geeigneter Enden, Auffüllen kohäsiver Enden, wie erforderlich, Behandlung mit alkalischer Phosphatase, um ungewollte Verknüpfungen zu ver-
20 meiden, 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
25 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). Über-
30 dies 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.

35

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
40 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
45 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
5 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 variierte Bank von Desaturase-Varianten durch kombinatorische Mutagenese auf Nukleinsäure-
10 ebene erzeugt und durch eine variierte Genbank kodiert. Eine variierte 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
15 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
20 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
25 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
30 et al., (1984) Science 198:1056; Ike et al. (1983) Nucleic Acids Res. 11:477).

Zusätzlich können Banken von Desaturase-Fragmenten zur Herstellung einer variierten Population von Desaturase-Fragmenten
35 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
40 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
45 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 Gen-
5 produkten 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
10 von Desaturase-Homologen erzeugt worden sind. Die am häufigsten
verwendeten Techniken zum Sichtung großer Genbanken, die einer
Analyse mit hohem Durchsatz unterworfen werden können, um-
fassen gewöhnlich das Klonieren der Genbank in replizierbare
Expressionsvektoren, Transformieren von geeigneten Zellen mit der
15 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-Muta-
genese (REM), eine neue Technik, die die Häufigkeit funktioneller
20 Mutanten in den Banken erhöht, kann in Kombination mit den
Sichtungstests zur Identifikation von Desaturase-Homologen ver-
wendet werden (Arkin und Yourvan (1992) Proc. Natl. Acad. Sci.
USA 89:7811-7815; Delgrave et al. (1993) Protein Engineering
6(3):327-331).

25

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
30 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
35 auf Funktionalität zu überprüfen.

Insbesondere für die Sichtung von Desaturaseaktivitäten
ist ein Sichtungsverfahren Voraussetzung, das PUFA-abhängig
Enzymaktivität(en) erfaßt. Bzgl. Desaturaseaktivitäten mit
40 Spezifität für PUFAs kann man in *Mucor*-Species, die durch
bekannte Transformationsverfahren mit gewünschten Genkonstrukten
transformierbar sind, die Toxizität von Arachidonsäure in An-
wesenheit eines toxischen Metaboliten (hier: Salicylsäure oder
Salicylsäurederivate) nutzen (Eroshin et al., *Mikrobiologiya*,
45 Vol. 65, No.1 1996, Seiten 31-36), um eine wachstumsbasierte
Erstsichtung durchzuführen. Resultierende Klone können dann einer
Analyse ihrer Lipidinhaltstoffe mittels Gaschromatographie und

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Massenspektroskopie unterzogen werden, um Edukte und Produkte in Art und Menge zu erfassen.

Bei einer weiteren Ausführungsform können Tests auf Zellbasis
5 zur Analyse einer variierten Desaturase-Bank unter Verwendung von weiteren im Fachgebiet bekannten Verfahren ausgenutzt werden.

E. Erfindungsgemäße Verwendungen und Verfahren

- 10 Die hier beschriebenen Nukleinsäuremoleküle, Proteine, Protein-homologen, 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
15 *Phaeodactylum tricornerum* verwandt sind, Identifikation und Lokalisierung von *Phaeodactylum tricornerum*-Sequenzen von Interesse, Evolutionsstudien, Bestimmung von Desaturase-Proteinbereichen, die für die Funktion notwendig sind, Modulation einer Desaturase-Aktivität; Modulation des Stoffwechsels einer oder
20 mehrerer Zellmembrankomponenten; Modulation des Transmembran-transportes 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
25 zunächst zur Identifikation eines Organismus als *Phaeodactylum tricornerum* oder als naher Verwandter davon verwendet werden. Sie können auch zur Identifikation des Vorliegens von *Phaeodactylum tricornerum* oder eines Verwandten davon in einer Mischpopulation von Mikroorganismen verwendet werden. Die Erfindung stellt die
30 Nukleinsäuresequenzen einer Reihe von *Phaeodactylum tricornerum*-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 tricornerum* -Gens oder von
35 Teilen davon überspannt, das für diesen Organismus einzigartig ist, kann man bestimmen, ob dieser Organismus vorliegt. *Phaeodactylum tricornerum* selbst werden zur kommerziellen Produktion mehrfach ungesättigter Säuren verwendet und eignen darüber hinaus zur PUFA-Produktion auch in anderen Organismen insbesondere wenn
40 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.
45 Dies ist nicht nur zur Kartierung des Genoms, sondern auch für funktionelle *Phaeodactylum tricornerum*-Proteinen geeignet. Zur Identifikation des Genombereichs, an den ein bestimmtes DNA-

bindendes Protein von *Phaeodactylum tricornerum* bindet, könnte das *Phaeodactylum tricornerum*-Genom zum Beispiel gespalten werden und die Fragmente mit dem DNA-bindenden Protein inkubiert werden. Diejenigen, die das Protein binden, können zusätzlich mit den

5 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 tricornerum* und erleichtert, wenn dies mehrmals mit unterschied-

10 lichen 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

15 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 er-

20 findungsgemäßen 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-Verwandtschaftsgrad der Organismen

25 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-Unter-

30 suchungen wertvoll und kann einen Hinweis darauf geben, wieviel Mutagenese das Protein tolerieren kann, ohne die Funktion zu verlieren.

Die Manipulation der erfindungsgemäßen Desaturase-Nukleinsäure-

35 moleküle 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. Ver-

40 besserte 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

45 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

5 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

10 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

15 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

20 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

25 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

30 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.

40

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

45 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.

10 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 Fein-

15 chemikalie 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

20 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

25 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

30 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

35 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 über-

40 leben 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.

45 Die vorstehend genannten Mutagenesestrategien für Desaturasen, die zu erhöhten Ausbeuten einer Feinchemikalie führen sollen, sollen nicht beschränkend sein; Variationen dieser Strategien

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- 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
- 5 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
- 10 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.
- 15 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
- 20 Vektor umfasst, welche ein Polypeptid kodieren, das C₁₈-, C₂₀- oder C₂₂-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
- 25 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, Glyco-
- 30 lipide, 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
- 35 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.
- 40 Vorzugsweise sind die durch dieses Verfahren produzierten PUFAs C₁₈- oder C₂₀₋₂₂-Fettsäuremoleküle mit mindestens zwei Doppelbindungen im Fettsäuremolekül, vorzugsweise drei, vier, bei Kombination mit einer weiteren Elongasen und einer Δ-4 Desaturase
- 45 fünf oder sechs Doppelbindungen. Diese C₁₈- oder C₂₀₋₂₂-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.

10 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 eine Verringerung der Desaturaseaktivität anzeigt, daß der Kandidatenstoff ein Antagonist ist.

30 Der genannte Kandidatenstoff kann ein chemisch synthetisierter oder mikrobiologisch produzierter Stoff sein und z.B. in Zell-extrakten 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 reprimierend. 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.

45 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
5 oder ein Fragment davon, wie sie hierin beschrieben werden, der die Aktivität der erfindungsgemäßen Desaturasen inhibiert.

Bei einem Aspekt betrifft die vorliegende Erfindung ein Anti-
körper, der spezifisch den erfindungsgemäßen oben beschriebenen
10 Agonisten oder Antagonisten erkennt bzw. bindet.

Ein weiterer Aspekt betrifft eine Zusammensetzung, die den Anti-
körper, den nach dem erfindungsgemäßen Verfahren identifizierten
Stopp oder das Antisense-Molekül umfaßt.

15

In einer weiteren Ausführungsform betrifft die vorliegende
Erfindung ein Kit, umfassend die erfindungsgemäße Nukleinsäure,
das erfindungsgemäße Genkonstrukt, die erfindungsgemäße Amino-
säuresequenz, das erfindungsgemäße Antisense-Nukleinsäuremolekül,
20 den erfindungsgemäßen Antikörper und/oder Zusammensetzung, einen
Antagonisten oder Agonisten, der nach dem erfindungsgemäßen Ver-
fahren hergestellt wurde, und/oder erfindungsgemäße Öle, Lipide
und/oder Fettsäuren oder eine Fraktion davon. Ebenso kann das
Kit die erfindungsgemäßen Wirtszellen, Organismen, Pflanzen
25 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, bei-
spielsweise mit oder in Puffern oder anderen Lösungen verpackt
30 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
35 Kit kann für jede der hierin beschriebenen Methoden und Aus-
führungsformen 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
40 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
45 zitierten Literaturstellen, Patentanmeldungen, Patente und

veröffentlichten Patentanmeldungen ist hier durch Bezugnahme aufgenommen.

Beispielteil

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Beispiel 1: Allgemeine Verfahren

a) Allgemeine Klonierungsverfahren:

- 10 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
- 15 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,
- 20 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

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- 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
- 30 von reinem pyrogenfreiem Wasser, im nachstehenden Text als H₂O 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
- 35 (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 ange-
- 40 geben, wurden sie nach den Anweisungen des Herstellers verwendet.

45

c) Zellmaterial

Die erfindungsgemäßen isolierten Nukleinsäuresequenzen sind im Genom eines *Phaeodactylum tricornerutum* UTEX646-Stammes enthalten, 5 der über die Algensammlung der University of Texas, Austin verfügbar ist.

Phaeodactylum tricornerutum wurde bei 25°C mit einem Licht/Dunkel Rhythmus von 14:10 Stunden bei 22°C und 35 microEinstein (ent- 10 spricht micromol Photonen pro Quadratmeter und Sekunde) in Glasröhren kultiviert, die von unten mit Luft begast wurden.

Als Kulturmedium für *Phaeodactylum tricornerutum* wurde das f/2 Kulturmedium mit 10 % organischen Medium nach Guillard, R.R.L. 15 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

20 995,5 ml Seewasser (artifiziert)
1 ml NaNO₃ (75 g/l), 1 ml NaH₂PO₄ (5 g/l), 1 ml Spurenelemente-
lösung, 1 ml Tris/Cl pH 8,0, 0.5 ml f/2 Vitaminlösung

Spurenelementelösung: Na₂EDTA (4,36 g/l), FeCl₃ (3,15 g/l),
25 Primäre Spurenelemente: CuSO₄ (10 g/l), ZnSO₄ (22 g/l), CoCl₂
(10 g/l), MnCl₂ (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
30 (3 g/l), Bacto-Trypton (4 g/l), Hefe-Extrakt (2 g/l)

Beispiel: 2 Isolierung von Gesamt-DNA aus *Phaeodactylum tri-*
cornerutum UTEX646 für Hybridisierungsexperimente

35 Die Einzelheiten der Isolierung von Gesamt-DNA betreffen die Auf-
arbeitung von Pflanzenmaterial mit einem Frischgewicht von einem
Gramm.

CTAB-Puffer: 2 % (Gew./Vol.) N-Acetyl-N,N,N-trimethylammonium-
40 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.

45 *Phaeodactylum tricornerutum*-Zellmaterial wurde unter flüssigem
Stickstoff in einem Mörser verrieben, so dass ein feines Pulver
erhalten wurde, und in 2 ml-Eppendorfgefäße überführt. Das ge-

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gefrorene Pflanzenmaterial wurde dann mit einer Schicht von 1 ml
 Zersetzungspuffer (1 ml CTAB-Puffer, 100 ml N-Laurylsarkosin-
 Puffer, 20 ml β -Mercaptoethanol und 10 ml Proteinase K-Lösung,
 10 mg/ml) überschichtet und eine Stunde unter kontinuierlichem
 5 Schütteln bei 60°C inkubiert. Das erhaltene Homogenat wurde
 in zwei Eppendorfggefäße (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
 10 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
 15 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 Waschschrift mit 70 % Ethanol wurde die
 DNA getrocknet und anschließend in 50 ml H₂O + RNase (50 mg/ml
 Endkonzentration) aufgenommen. Die DNA wurde über Nacht bei 4°C
 20 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*

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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
 30 et al., 1994, Mol. Gen. Genet., 244:352-359) gewonnen werden.

RNA Isolierung aus *Phaeodactylum tricornutum*:

Tiefgefrorene Algenproben (- 70°C) wurden in einem eiskaltem
 35 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 H₂O
 aufgefüllt und der pH auf 8,5 eingestellt) und 4 Volumen Phenol
 40 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
 45 extrahiert.

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Das erhaltene Volumen der wässrigen Phase wurde mit 1/20 Vol 4 M Na-Acetat (pH 6) und 1 Vol Isopropanol (eiskalt) versetzt 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 Waschschrift 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[®] (Dyna, 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 Formaldehydhaltigen 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 Reverse 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.

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Beispiel 5: DNA-Sequenzierung und Computeranalyse

cDNA-Banken, wie im Beispiel 4 beschrieben, wurden zur DNA-Sequenzierung nach Standardverfahren, insbesondere durch das

10 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-

15 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

20 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:

25 5'--CAGGAAACAGCTATGACC-3'
 5'--CTAAAGGGAACAAAAGCTG-3'
 5'--TGTAACGACGGCCAGT-3'

Die Sequenzen wurden unter Verwendung des Standard-Softwarepakets EST-MAX, das kommerziell von Bio-Max (München, Deutschland)

30 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

35 PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.). Zwei Sequenzen aus *Phaeodactylum tricornerutum* mit Homologien zur Suchsequenz aus *Physcomitrella patens* wurden eingehender charakterisiert.

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Beispiel 5a: Isolation von Desaturasen aus *Phaeodactylum tri-cornutum* über Polymerase Kettenreaktion mithilfe degenerierter Oligonukleotide:

- 5 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-Buchstaben-code die Aminosäure dargestellt, von der die Basenkombination
- 10 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 Basen-
- 15 triplett 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:

20 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

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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

- 30 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

35 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

40 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

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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			
5	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		
10	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		
15	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
20	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	
25	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

80

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
5 gekennzeichnet und konnten erfolgreich zur Isolierung eines
Desaturasefragmentes genutzt werden. Das resultierende Fragment
konnte durch Sequenzierung verifiziert werden und zeigte Homo-
logien zu einer Desaturase mit der Genbank Accession Nr. T36617
aus *Streptomyces coelicolor*. Die Homologie wurde mithilfe des
10 BLASTP Programmes erhalten. Der Vergleich ist in Figur 4 dar-
gestellt. Es ergaben sich Identitäten von 34 % und eine Homo-
logie von 43 % zu Sequenz T36617. Das DNA-Fragment wurde gemäß
Beispiel 7 in einem Hybridisierungsexperiment zur Isolierung
eines Vollängens nach Standardbedingungen erfindungsgemäß
15 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
20 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 Ver-
gleich mit einer in WO 98 46763 beschriebenen Gensequenz wurde
25 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 Substratspezifität. Dies wird auch dadurch deutlich, dass
30 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.

35 Beispiel 6: Identifizierung von DNA Sequenzen codierend für
Desaturasen aus *Phaeodactylum tricornutum*

Die Vollängensequenz der Δ -6-Acyl Lipid Desaturase Pp_des6
AJ222980 (NCBI Genbank Accession Nr.) aus dem Moos Physco-
40 mitrella 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 Suchalgorithmus
eingesetzt.

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81

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 5 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 10 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 15 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 20 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, 25 Acetylasen 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, 30 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.

35

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. 45 Der Vergleich wurde mit der BLOSUM62 Austauschmatrix für Amino-

82

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.

5

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
 10 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 Polypeptidsequenz ist in SEQ ID NO: 6 dargestellt.

15 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 Polypeptidsequenz ist in SEQ ID NO: 12
 20 dargestellt.

In Tabelle 2 sind die Identitäten und Homologien erfindungsgemäßer Desaturasen untereinander und mit der Desaturase aus *Physcomitrella patens* und *Mortierella alpina* dargestellt. Die
 25 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
 30 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.

35

Tabelle 2:

Homologie / Identität in %	Suchsequenz Pp_des6	Suchsequenz Ma_des12
40 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

45 n.d. = nicht durchgeführt

83

Mithilfe des Algorithmus 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 Sequenz-
 5 homologie bzw. Identität identifiziert. Die Ergebnisse sind in folgender Tabelle 2A dargestellt.

Tabelle 2A: Homologe mit den höchsten Sequenzhomologien bzw Identitäten zu erfindungsgemäßen Polypeptidsequenzen
 10 aus SEQ ID NO. 2, 4, 6 oder 12

Homologie / Identität (%)	Suchsequenz PT001070010R	Suchsequenz PT001072031R	Suchsequenz PT001078032R	Suchsequenz Pt_des6
15 L26296: Fad2 A. thaliana	50 % / 37 %	n.d.	n.d.	n.d.
U86072 Petro- selinum crispum Fad2	n.d.	51/40	n.d.	n.d.
20 AL358652 L. major putative desaturase	n.d.	n.d.	45/30	n.d.
25 AB020032 M. alpina delta 6 desaturase	n.d.	n.d.	n.d.	53/38

Beispiel 7: Identifikation von Genen mittels Hybridisierung

30 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: Ins-
 35 besondere 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
 40 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 Waschschr
 45 mittels radioaktiver (³²P-) Nicktranskription (High Prime, Roche,

Mannheim, Deutschland) hergestellt. Die Signale werden mittels Autoradiographie nachgewiesen.

Partiell homologe oder heterologe Gene, die verwandt, aber nicht
5 identisch sind, lassen sich analog zum oben beschriebenen Ver-
fahren 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.

10

Die Isolierung von Gensequenzen, die nur zu einer einzelnen
Domäne von beispielsweise 10 bis 20 Aminosäuren Homologien auf-
weisen, lässt sich unter Verwendung synthetischer, radioaktiv
markierter Oligonukleotidsonden durchführen. Radioaktiv markierte
15 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 Nick-
20 transkription radioaktiv markiert. Die Hybridisierung erfolgt
gewöhnlich bei niedrig-stringenten Bedingungen unter Verwendung
hoher Oligonukleotidkonzentrationen.

Oligonukleotid-Hybridisierungslösung:

25

6 x SSC

0,01 M Natriumphosphat

1 mM EDTA (pH 8)

0,5 % SDS

30 100 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-T_m oder bis auf Raum-
35 temperatur (bedeutet RT = ~ 23°C in allen Experimenten, wenn nicht
anders angegeben) gesenkt, gefolgt von Waschschritten und Auto-
radiographie. Das Waschen wird mit extrem niedriger Stringenz
durchgeführt, zum Beispiel 3 Waschschrritte unter Verwendung von
4 X SSC. Weitere Einzelheiten sind wie von Sambrook, J., et al.
40 (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.

45

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Beispiel 8: Identifikation von Zielgenen durch Sichtung von Expressionsbanken mit Antikörpern

Es wurden cDNA-Sequenzen zur Herstellung von rekombinantem
 5 Protein zum Beispiel in *E. coli* verwendet (z.B. Qiagen QIAexpress pQE-System). Die rekombinanten Proteine wurden dann gewöhnlich über Ni-NTA-Affinitätschromatographie (Qiagen) affinitäts-
 gereinigt. Die rekombinanten Proteine wurden dann zur Herstellung
 spezifischer Antikörper beispielsweise unter Verwendung von
 10 Standardtechniken zur Immunisierung von Kaninchen verwendet. Anschließend wurden die Antikörper dann unter Verwendung einer Ni-NTA-Säule, die mit rekombinantem Antigen vorgesättigt wird, affinitäts-
 gereinigt, wie von Gu et al., (1994) *BioTechniques* 17:257-262 beschrieben. Der Antikörper kann dann zur Durch-
 15 musterung 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).

20

Beispiel 9: Transformation von *Agrobacterium*

Die *Agrobacterium*-vermittelte Pflanzentransformation kann zum
 Beispiel unter Verwendung des GV3101- (pMP90-) (Koncz und Schell,
 25 *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).

30

Beispiel 10: Pflanzentransformation

Die *Agrobacterium*-vermittelte Pflanzentransformation kann unter
 Verwendung von Standard-Transformations- und Regenerations-
 35 Techniken 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:
 40 CRC Press, 1993, 360 S., ISBN 0-8493-5164-2).

Beispielsweise kann Raps mittels Kotyledonen- oder Hypokotyl-
 transformation transformiert werden (Moloney et al., *Plant*
Cell 8 (1989) 238-242; De Block et al., *Plant Physiol.* 91 (1989)
 45 694-701). Die Verwendung von Antibiotika für die *Agrobacterium*-
 und Pflanzenselektion hängt von dem für die Transformation
 verwendeten binären Vektor und *Agrobacterium*-Stamm ab. Die

86

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).

20 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).

40 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 Pflanze 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 Polymerasekettenreaktion isoliert und mit flankierenden Sequenzen nach Wahl auf Basis von synthetischen Oligonukleotiden maßgeschneidert.

Folgende Oligonukleotide können beispielsweise verwendet werden:

- USP1 vorne: CCGGAATTCCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA
 USP2 vorne: CCGGAATTCCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA
 USP3 vorne: CCGGAATTCCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA
 5 USP1 hinten:AAAAGTGCAGGCGGCCGCCACCGCGGTGGGCTGGCTATGAAGAAATT
 USP2 hinten:CGCGGATCCGCTGGCTATGAAGAAATT
 USP3 hinten:TCCCCGGGATCGATGCCGGCAGATCTGCTGGCTATGAAGAAATT
 OCS1 vorne: AAAAGTGCAGTCTAGAAGGCCTCCTGCTTTAATGAGATAT
 OCS2 vorne: CGCGGATCCGATATCGGGCCCGCTAGCGTTAACCTGCTTTAATGAGATAT
 10 OCS3 vorne: TCCCCGGGCCATGGCCTGCTTTAATGAGATAT
 OCS1 hinten:CCCAAGCTTGGCGCGCCGAGCTCGAATTCGTCGACGGACAATCAGTAAATTGA
 OCS2 hinten:CCCAAGCTTGGCGCGCCGAGCTCGAATTCGTCGACGGACAATCAGTAAATTGA
 OCS3 hinten:CCCAAGCTTGGCGCGCCGAGCTCGTCGACGGACAATCAGTAAATTGA

- 15 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 Empfänger-
 20 plasmid 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.

- 25 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 Expressions-
 30 kassetten enthaltenden Fragmente werden ligiert und in E. coli XLI blue MRF transformiert. Es wird nach Vereinzellung ampicillinresistenter Kolonien DNA präpariert und per Restriktionsanalyse solche Klone identifiziert, die zwei Expressionskassetten enthalten. Die XhoI/Sali Ligation kompatibler Enden hat dabei die
 35 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
 40 werden ligiert und in E. coli XLI blue MRF transformiert. Es wird nach Vereinzellung 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 ge-
 45 wünschter DNA genutzt werden kann und in Tabelle 3 beschrieben wird und zudem noch weitere Expressionskassetten aufnehmen kann.

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Diese enthalten folgende Elemente:

Tabelle 3

	pUC19-Derivat	Schnittstellen vor dem USP Promotor	Multiple Klonierungs-Schnittstellen	Schnittstellen hinter dem OCS-Terminator
5	pUT1	EcoRI/AscI/ SacI/XhoI	BstXI/NotI/ PstI/XbaI/StuI	Sall/EcoRI/ SacI/AscI/ HindIII
	pUT2	EcoRI/AscI/ SacI/XhoI	BamHI/EcoRV/ ApaI/NheI/ HpaI	Sall/EcoRI/ SacI/AscI/ HindIII
10	pUT3	EcoRI/AscI/ SacI/XhoI	BglII/NaeI/ ClaI/SmaI/NcoI	Sall/SacI/ AscI/HindIII
	pUT12 Doppel-expressionskassette	EcoRI/AscI/ SacI/XhoI	BstXI/NotI/ PstI/XbaI/StuI Und BamHI/EcoRV/ ApaI/NheI/ HpaI	Sall/EcoRI/ SacI/AscI/ HindIII
15	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	Sall/SacI/AscI/HindIII

20

Weiterhin lassen sich wie beschrieben und wie in Tabelle 4 näher spezifiziert weitere Multiexpressionskassetten mithilfe des

- i) USP-Promotors oder mithilfe des
- 25 ii) ca. 700 Basenpaare 3'-Fragmentes des LeB4-Promotors oder mithilfe des
- iii) DC3-Promotors erzeugen und für samenspezifische Genexpression einsetzen.
- 30 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
- 35 Promotoren aufgebaut werden.

40

45

Tabelle 4: Multiple Expressionskassetten

	Plasmidname des pUC19-Derivates	Schnittstellen vor dem jeweiligen Promotor	Multiple Klonierungs-Schnittstellen	Schnittstellen hinter dem OCS-Terminator
5	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
10	pLeBT (pUC19-mit LeB4(700)-OCS)	EcoRI/AscI/SacI/XhoI	(3) BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/HindIII
15	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
20	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)

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Analog lassen sich weitere Promotoren für Multigenkonstrukte erzeugen insbesondere unter Verwendung des

- 30 a) 2,7 kB Fragmentes des LeB4-Promotors oder mithilfe des
b) Phaseolin-Promotors oder mithilfe des
c) konstitutiven v-ATPase cl-Promotors.

35 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.

- 40 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.

45 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

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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
5 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

- 10 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
15 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.

20

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

- 25 aus Phaeodactylum (Pt_des5) über BglIII 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.

30

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

- 35 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.

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Tabelle 5: Kombinationen von Desaturasen und Elongasen

	Gen Plasmid	Δ -6-Desaturase	Δ -5-Desaturase	Δ -6-Elongase	
5	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
10	7	PBARAI	Pt_des6	Pt_des5	Pp_PSE1

Plasmide 1 bis 5 sind pUC Derivate, Plasmide 6 bis 7 sind binäre Pflanzentransformationsvektoren

- 15 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)
- 20 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)
- 25 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.
- 30 iii) Transfer von Expressionskassetten in Vektoren zur Transformation von *Agrobacterium tumefaciens* und zur Transformation von Pflanzen
- 35 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
- 40 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.

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Beispiel 12: *In vivo*-Mutagenese

Die *in vivo*-Mutagenese von Mikroorganismen kann mittels Passage der Plasmid- (oder einer anderen Vektor-) DNA durch *E. coli* 5 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* 10 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 15 mutierter DNA-Moleküle in Pflanzen erfolgt vorzugsweise nach Selektion und Test der Mikroorganismen. Transgene Pflanzen werden nach verschiedenen Beispielen im Beispielteil dieses Dokumentes erzeugt.

20 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 25 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) 30 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 35 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 40 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 45 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
5 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
10 3 Stunden bei 68°C unter Verwendung von Hybridisierungspuffer (10 % Dextransulfat Gew./Vol., 1 M NaCl, 1 % SDS, 100 mg Heringsperma-DNA) vorhybridisiert. Die Markierung der DNA-Sonde mit dem Highprime DNA labeling-Kit (Roche, Mannheim, Deutschland) erfolgte während der Vorhybridisierung unter Verwendung von
15 alpha-³²P-dCTP (Amersham, Braunschweig, Deutschland). Die Hybridisierung wurde nach Zugabe der markierten DNA-Sonde im gleichen Puffer bei 68°C über Nacht durchgeführt. Die Waschschr

20 geführt. 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
25 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
30 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
35 gewünschten, in der Zelle vorliegenden mutierten Proteins an.

Beispiel 14: Analyse der Auswirkung der rekombinanten Proteine auf die Produktion des gewünschten Produktes

40 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)
45 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-

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- techniken sind dem Fachmann bekannt und umfassen Spektroskopie, Dünnschichtchromatographie, Färbeverfahren verschiedener Art, enzymatische und mikrobiologische Verfahren sowie analytische Chromatographie, wie Hochleistungs-Flüssigkeitschromatographie
- 5 (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:
- 10 "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)
- 15 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).
- 20 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
- 25 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:
- 30 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 ana-
- 35 lysieren, 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
- 40 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.
- 45 Stanbury, Hrsgb., IRL Press, S. 103-129; 131-163 und 165-192

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(ISBN: 0199635773) und darin angegebenen Literaturstellen beschrieben.

Ein Beispiel ist die Analyse von Fettsäuren (Abkürzungen: FAME, 5 Fettsäuremethylester; GC-MS, Gas-Flüssigkeitschromatographie-Massenspektrometrie; TAG, Triacylglycerin; TLC, Dünnschichtchromatographie).

Der unzweideutige Nachweis für das Vorliegen von Fettsäure- 10 produkten 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 15 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 20 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- 25 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 30 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.

35 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) 40 gezeigt werden.

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Expressionskonstrukte in heterologen mikrobiellen Systemen

Stämme, Wachstumsbedingungen und Plasmide

- 5 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,
- 10 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,
- 15 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)
- 20 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*

- 25 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
- 30 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.
- 35 (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,
- 40 in den das Fragment kloniert werden soll und mit dessen Hilfe die Genexpression in Mikroorganismen oder Pflanzen erfolgen soll.

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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

30 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

40 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.

99

$\Delta 12$ 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

5 $\Delta 12$ 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
10 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
15 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
20 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.

25

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-
30 Transformanden und eine pYES2-Transformande zur weiteren Anzucht
und funktionellen Expression ausgewählt. Bei pYES6-Derivaten
wurde Blastocidin als Antimetabolit verwendet. Im Fall von
Coexpressionen auf Basis von pYES2 und pYES6 wurde auf Minimal-
medium mit Blastocidin selektiert.

35

Funktionelle Expression einer Desaturaseaktivität in Hefe

Vorkultur

40 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 (OD₆₀₀) von 1,5 bis 2 erreicht wurde. Wurde als Vektor
pYES6 verwendet, so wurde zusätzlich auf Blastocidin als Anti-
45 metabolit selektioniert.

100

Hauptkultur

Für die Expression wurden 20 ml CMDum-Flüssigmedium ohne Uracil aber mit 2 % Raffinose und 1 % (Vol./Vol.) Tergitol NP-40

- 5 mit Fettsäuresubstraten auf eine Endkonzentration von 0,003 % (Gew./Vol.) angereichert. Die Medien wurden mit den Vorkulturen auf eine OD₆₀₀ von 0,05 angeimpft. Die Expression wurde bei einer OD₆₀₀ von 0,2 mit 2 % (Gew./Vol.) Galaktose für 16 Std. induziert, wonach die Kulturen eine OD₆₀₀ von 0,8-1,2 geerntet wurden.

10

Fettsäureanalyse

- Die Gesamt-Fettsäuren wurden aus Hefekulturen extrahiert und mittels Gaschromatographie analysiert. Davon wurden Zellen von 5
- 15 ml Kultur mittels Zentrifugation (1000 x g, 10 min, 4°C) geerntet und einmal mit 100 mM NaHCO₃, 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 H₂SO₄ und 2 % (Vol./Vol.)
- 20 Dimethoxypropan für 1 Std. bei 80°C behandelt. Die FAMES wurden zweimal mit 2 ml Petrolether extrahiert, einmal mit 100 mM NaHCO₃, pH 8,0, und einmal mit destilliertem Wasser gewaschen und mit Na₂SO₄ getrocknet. Das organische Lösungsmittel wurde unter einem Argonstrom verdampft, und die FAMES wurden in 50 Mikrol Petrol-
- 25 ether gelöst. Die Proben wurden auf einer ZEBRON-ZB-Wax-Kapillarsäule (30 m, 0,32 mm, 0,25 Mikrom; 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
- 30 (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.

35

Expressionsanalyse

- Die Verhältnisse der zugegebenen und aufgenommenen Fettsäuresubstrate wurden ermittelt und so Quantität und Qualität der
- 40 Desaturasereaktion gemäß Tabelle 6, Tabelle 7 und Tabelle 8 erfasst.

Ergebnis der Expression einer *Phaeodactylum tricornutum* Δ-6-Acyl Lipid Desaturase in Hefe:

45

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Tabelle 6

Fettsäure	pYes2	pYes2-Ptd6 gefüttert mit		
		-	+18:2	+18:3
5 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	-	-
10 18:2Δ9,12	-	-	33,4	-
18:3Δ6,12,15	-	-	4,9	-
18:3Δ9,12,15	-	-	-	43,1
18:4Δ6,9,12,15	-	-	-	2,3

15 Die Angaben stellen Mol-% entsprechender cis-Fettsäuren dar.

Ergebnis der Expression einer Phaeodactylum tricornutum Δ-5-Acyl Lipid Desaturase in Hefe:

20 Tabelle 7

Fett- säure	pYES2		pYES_PtD5-Konstrukt gefüttert mit						
	Leer	Kon- trolle	18:2	18:3	20:1 Δ8	-20:1 Δ11	20:2 Δ11,14	20:3 Ω3	20:3 Ω6
25 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
30 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	-	-	-
35 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
40 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.

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Aus weiteren Fütterungsversuchen wurde gefunden, dass C18:1 Δ 9 in der Anwesenheit von C18:2 Δ 9,11 oder C18:3 Δ 9,12,15 oder C20:1 Δ 8 Fettsäuren nicht desaturiert wurde während in Anwesenheit von C20:1 Δ 11, C20:2 Δ 11,14 und C20:3 Δ 8,11,14 auch C18:1 desaturiert wird. Ebenfalls keine Desaturierung erfolgte in Anwesenheit von C20:3 Δ 8,11,14.

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 tricornerutum* auf Vollmedium mit Blastocidin wurde gefunden, dass C20:4 Δ 8,11,14,17 als Substrat der Δ -5-Desaturase mit 20 % Umsatzrate ebenso gut umgesetzt wurde wie C20:3 Δ 8,11,14. 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 Δ 8,11,14 zu C20:4 Δ 5,8,11,14 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, Stearidon-säure, Arachidonsäure, Eicosapentaensäure etc.) können zur detaillierteren Bestätigung der Substratspezifität und -Selektivität dieser Desaturasen durchgeführt werden.

Tabelle 8: Ergebnis der Coexpression einer *Phaeodactylum tricornerutum* Δ -5-Acyl Lipid Desaturase und einer Δ -6 Elongase aus Moos in Hefe auf Basis der Expressionsvektoren pYes2 und pYes6

35	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
16:1 Δ 9	27,7	29,2	27,5	29,0
18:0	5,6	6,3	5,7	6,4
40 18:1 Δ 9	17,1	30,8	27,4	31,6
18:3 Δ 6,9,12	7,60	-	7,8	-
18:4 Δ 6,9,12,15	-	6,71	-	6,4
20:3 Δ 8,11,14	15,92	-	13,55	-
45 20:4 Δ 5,8,11,14	-	-	1,31	-
20:4 Δ 8,11,14,17	-	11,4	-	10,31
20:5 Δ 5,8,11,14,17	-	-	-	0,53

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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 Arachidon-
5 säure bzw. Eicosapentaensäure mithilfe erfindungsgemäßer Sequenzen zu produzieren.

Die Fragmentierungsmuster und Massenspektren von DMOX-Derivaten von Standards als auch den Peakfraktionen per GC identifizierter
10 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.

15 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
20 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
25 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
30 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.

35 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
40 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
45 gereinigte Produkt kann durch Filtration oder Ultrafiltration konzentriert und bei einer Temperatur aufbewahrt werden, bei der die Stabilität des Produktes maximal ist.

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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
5 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), spektro-
10 skopische 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;
15 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
20 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

25

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.

30

35

40

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Patentansprüche

1. Verfahren zur Herstellung von Fettsäureestern mit einem
5 erhöhtem Gehalt an mehrfach ungesättigten Fettsäuren mit
mindestens zwei Doppelbindungen dadurch gekennzeichnet, daß
man mindestens eine Nukleinsäuresequenz ausgewählt aus der
Gruppe
- 10 a) einer Nukleinsäuresequenz mit der in SEQ ID NO: 1,
SEQ ID NO: 3, SEQ ID NO: 5 oder SEQ ID NO: 11 dar-
gestellten Sequenz,
- b) Nukleinsäuresequenzen, die aufgrund des degenerierten
15 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,
- 20 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 Aminosäure-
25 sequenzen 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
30 isoliert.
2. Verfahren nach Anspruch 1, wobei die durch das Verfahren
hergestellten Fettsäureester mehrfach ungesättigte C₁₈-, C₂₀-
oder C₂₂-Fettsäuremoleküle mit mindestens zwei Doppelbindungen
35 im Fettsäureester enthalten.
3. Verfahren nach Anspruch 1 oder 2, wobei die C₁₈-, C₂₀- oder
C₂₂-Fettsäuremoleküle aus dem Organismus in Form eines Öls,
Lipids isoliert werden.
40
4. Verfahren nach den Ansprüchen 1 bis 3, wobei der Organismus
ein Mikroorganismus, ein Tier oder eine Pflanze ist.

45

Zeichn.

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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 C₁₈-, C₂₀- oder C₂₂-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 Biosynthesegene des Fettsäure- oder Lipidstoffwechsels enthalten sind.

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13. Nukleinsäurekonstrukt nach Anspruch 11 oder 12, wobei als Biosynthesegen des Fettsäure- oder Lipidstoffwechsels im Nukleinsäurekonstrukt ein Gen ausgewählt aus der Gruppe
5 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,
10 Hydroperoxid-Lyasen oder Fettsäure-Elongase(n) enthalten ist.
14. Vektor enthaltend eine Nukleinsäuresequenz gemäß Anspruch 8 oder ein Nukleinsäurekonstrukt gemäß Anspruch 11.
- 15 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
20 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
25 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
30 von transgenen Pflanzen.
19. Antikörper, der spezifisch ein Polypeptid, das von einer der Nukleinsäuren gemäß Anspruch 8 codiert wird, bindet.
- 35 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.
40
22. Öl-, Lipid- oder Fettsäurezusammensetzung, die Öl, Lipide oder Fettsäuren oder eine Fraktion davon gemäß Anspruch 21 enthalten.
45

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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.
- 5
24. Verfahren zur Identifikation eines Antagonisten oder Agonisten von Desaturasen, umfassend
- 10 a) in Kontaktbringen der Zellen, die das Polypeptid der vorliegenden Erfindung exprimieren, mit einem Kandidatenstoff;
- b) Testen der Desaturase-Aktivität;
- 15 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
- 20 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.
- 25
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.
- 30
27. Zusammensetzung, enthaltend den Antikörper gemäß Anspruch 19, das Antisense-Nukleinsäurekonstrukt gemäß Anspruch 20 oder einen Antagonisten oder Agonisten identifiziert gemäß Anspruch 24.
- 35
- 40
- 45

Figur 1: Polypeptidvergleich der Codierregionen von Pp_des 6 (obere Reihe) mit der EST-Sequenz von PT001078032R (untere Reihe)

```

398 WKPLVWMAVTELMMSGMLLGFVFLSHNGMEVYNSSKEFVSAQI-----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 CTYGGFSLSGCFTGGLNFQVEHHLFPRMSSAWYXYIAPKVREICAKHGVHY 113
    
```

Figur 2: Polypeptidvergleich von Codierregionen der Ma_des12 (obere Sequenz) mit PT001070010R

```

105 GVWVLAHECGHQSFSTSKTLNN 126
      G WVLAHECGH +FS .+++L +
533 GFWVLAHECGHGAFSKNRSLOD 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 Phoadactylum mit der Sequenz T36617 aus Streptomyces coelicolor (untere Reihe)

```

1   WWKNKHNGHHAVPNLHCSSAVAQDGDIDTPLLAWSVQQAQSYRELQADGKDSGLVKF 60
      WW++KH   HHA PN           +D DPDI   LL WS   QA++           +GL +
114 WWQDKHTRHHANFN-----TEDLDPDIGP-DLLVWSPDQARAA-----TGLPRL 156
61  MIRNQSYFYFPILLRLARLSWLNESFKCAFGLGAASENAALELKAKGLQYPLLEKAGILLH 120
      + R Q++ +FP+L L           E F           G A N L+ +A           L+ A +L H
157 LGRWQAFLLFFPLLTL-----EGFNLHVASGRAMANRRLKRRR-----LDGALLLAH 202
121 YAWMLTVSSGFGFRXXXXXXXXXXXXXXXXXXCGFLLAIVFGLGHNGMATYNADARPDFWKLQ 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-----LFTDLALGGLLNHQIEHHLFPS 291
    
```

Figur 4: Polypeptidvergleich von Codierregionen aus Pp_des6 (obere Reihe) verglichen mit Pt_des6 (untere Reihe)

```

51 KRLTSKKRVSESAAVQCISAEVQRNSSTQGTAEALAESVVKPTRRRSSQW 100
      . | | .
1 .....MGKGGDARASKG 12
      .
101 KKSTHPLS..EVAVHNKPSDCWIVVKNKVYDVSNFADDEHPGGSVISTYFG 148
      . :| || | | ||: ||||| |. |||||.|| |: |
13 STAARKISWQEVKTHASPEDAWIIHSNKVYDVSNW.HEHPGGAVIFTHAG 61
      .
149 RDGTDVFSSFHAASTWKILQDFYIGDV..ERVEPTPELL...KDFREMRA 193
      | ||:|..||| . ::. ||||:| | : : | :|:|.
62 DDMTDIFAAPHAPGSQSLMKKFYIGELLPETTGKEPQQIAFEKGYRDLRS 111
      .
194 LFLREQLFKSSKLYVVMKLLTNVAIFAASIAIICWSKTISAVLASACMMA 243
      : :|||.| :|| | |.|.||. ||. |: : :| |||| |:
112 KLIMMGFMFKSNKWFYVYKCLSNMAIWAACALVFYSDRFVWHLASAVMLG 161
      .
244 LCFQQCGWLSHDFLHNQVFETRWLNEVVGVIIGNAVLGFSTGWWKEKHNL 293
      ||| |||.|||||.||| | :. | || . |:| ||| |||
162 TFFQQSGWLAHDFLHHQVFTKRKHGDLGGLFWGNLMQGYSVQWKNKHNG 211
      .
294 HHAAPN.ECDQTY.QPIDEDIDTLPLIAWS.....KDILATVENKTF 334
      ||| || | | | ||||:|:| ||| ::| ..
212 HHAVPNLHCSSAVAQDGDPDIDTMPLLAWSVQQAQSYRELQADGKDSGLV 261
      .
335 R.ILQYQHLFFMGLLFFARGSWLFWSWR.....YTSTAVLSPVDR... 373
      : :. | |: :| || ||| |.: . | | :
262 KFMIRNQSYFFYPILLLARI,SWLNESFKCAFGLGAASENAALELKAKGLQ 311
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374 ..LLEKGTVLFHYFVFGTAC.YLLPGWKPLVWMAVTELMS.GMLLGFVF 419
      |||| |:| || | . . : . . .) | || || ||
312 YPLLEKAGILLHYAWMLTVSSGFGRFSAFYFLTATASCGFLLAIVF 361
      .
420 VLSHNGMEVYNSS..KEFVSAQIVSTRDIKG....NIFNDWFTGGLNRQ 462
      | |||| ||. :| |: .||.: | | ||| ||| |
362 GLGHNGMATYNADARPDFWKLQVTTTRNVTGGHGFPQAFVDWFCGGLQYQ 411
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463 IEHHLFPTMPRHNLNKIAPRVEVFCKKHGLVYEDVSIATGTCKVLKALKE 512
      ::|||||.:||||| | || |||. |. | : : || .|| |
412 VDHHLFPSLPRHNLAKTHALVESFCKEKGWVQYHEADLVDGTMEVLHHLGS 461
      .
513 VAEAAAEQHATTS.... 525
      || |
462 VAGEFVVDVFRDGPAM. 477

```

Figur 5: Polypeptidvergleich von Codierregionen aus Pp_des6 (obere Reihe) verglichen mit Pt_des5 (untere Reihe)

```

51 KRLTSKKRVSESAAVQCISAEVQRNSSTQGTAEALAESVVKPTRRRSSQW 100
                                     :|  |  .|...
1  .....MAPDADKLRQRQTAV 16
.
.
.
101 KKSTHPLSEVAVHNKPSDC.....WIVVKNKVYDVSNFADHEHPGGSVIS 144
    | |  . : . : : : :|} . .} :|}| |
17 AK..HNAATISTQERLCSLSSLKGEVVCIDGIIYDLQSF..DHPGGETIK 62
.
.
.
145 TYFGRDGTDFVSSPFAASTWKILQDF.YIGDVERVEPTPELLKDF.REM. 191
    : | } | : | | | : : | | . : | } | .
63 MFGCNDVTVQYKMIHPYHTEKHLEKMKRVGKVTDVFVCEYKFDTEFEREIK 112
.
.
.
192 RALFLREQLFKS.SKLYYVMKLLTNVAIFAASIAIICWSKT.ISAVLASA 239
    | .| . | | : : :|}| | | | .|}|
113 REVFKIVRRGKDFGTLGWFFRAFCYIAIF..FYLQYHWVTTGTSWLLAVA 160
.
.
.
240 CMMALCFQCCGWLSDHDFLHNQVFPETRWLNEVVGIVIGNAVLGFSTGWWKE 289
    .. . || | . |. : .. | :| :| | |. |
161 YGISQAMIGMN.VQHDANHGATSKRPWVNDMLG..LGADFIGGSKWLWQE 207
.
.
.
290 KHNHLHHAAPNECDQTYQPIDEDIDTLPLIAWSKDILATVENKTFRLRILQY 339
    .} |}| | : | : :| : .. | :|. | .:
208 QHWTHHAYTNHAEM..DP..DSFGAEPMLLFN.DYPLDHPARTWLH..RF 250
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.
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340 QHLFFMGLLFFARGSWLFWWR....YTSTAVLS...PVDRLLEKGTVL 381
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251 QAFFYMPVL...AGYWLSAVFNPQILDQLQKALSVGIRLDNAFIHSRRK 297
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382 FHYPW...FVG....TACYLLPG....WKPLVWMAVTELMGMLLGFVVFV 420
    : || :: | | | : . . . : | .|
298 YAVFWRAVYIAVNVIAPFYTNSSGLEWSWRVFGNIMLMGVAESLALAVLFS 347
.
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421 LSHN.....GMEVYNSSKEFVSAQIVSTRDIKGNIFNDWFTGGLN 460
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348 LSHNFESADRDPTAPLKKTGEPVDWFKTQ.VETSCTYGGFLSGCFTGGLN 396
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461 RQIEHHLFP'TMPRHNLNKIAPRVEVFCKKHGLVYEDVS.IATGTCKVLKA 509
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397 FQVEHHLF'PRMSSAWYPYIAPKVREICAKHGVHYAYYPWIHQNFLSTVRY 446
.
.
.
510 LKEVAEAAA.EQHATTS..... 525
    : | | | . |
447 MHAAGTGANWRQMARENPLTGRA. 469
    
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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 KEIRECIPAHCFERSGLRGLCHVAIDL TWASLL..FLAATQIDKFE..NP 85
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107 KDLRAVIPKDCFEPTAKSLGYLSVS.TMGTILCSVVGANLLSVLDPSNP 155
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      .
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86 LIRYLAWPVYWIMQGI VCTGVVWLAHECGHQSFSTSKTLNNTVGVWILHSM 135
   | : | | . | | |.||||||| |.|| .:.| . ||:|:|:|.
156 L.TWPLWAAYGAVTGT VAMGLWVLAHECGHGAFSKNRS LQDAVGYIIHSI 204
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      .
      .
136 LLVPYHSWRISHSKH KATGHMTKDQVFPKTRSQVGLPPKENAAA AVQE 185
   :|:| | |. | |. | |. | || : || . | | . | |
205 MLVPYFSWQRSHAVH HQYTNH MELGETHV PDRADKEG....EKSLALRQF 250
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      .
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186 EDMSVHLDEEAPIV TLFWMVIQFLFGWPAYLIMNASGQDYGRWTS HFHTY 235
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251 MLDSFGKDKGMKAYG GLQSFHLHIVGWPAYLLIGATGGPDRGMTNHFYP. 299
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      .
      .
236 SPIFEP....RNFF.....DIIISDLGVLAALGAL IYASMQLSLLTVTK 275
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300 NPLSTPTQPKKELFP GNWKEKVYQSDIGIAAVVGALIAWTAT SGLAPVMA 349
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      .
      .
276 YYIVPYL FVNFWLV LITFLQHTDPKLPHYREGAWNFORGALCTVDRSFGK 325
   | | : :| |||| |. ||||| |. ||: || :||| |:|| : |
350 LYGGPLIVINAWLV LYTWLQHTD TDVPHFSSDNHNFVK GALHTIDR PYDK 399
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      .
      .
326 .....FLDHMFHGI VHTHVAHHLFSQMPFYHAE EATYHLK KLLGEYYVYD 370
   :| : | | ||||| |. | | |: || :| | |. ||
400 LDPWGIIDFLH HKIGTTHVAH HFDSTIPHYKAQIATDAI KAKFPEVYLYD 449
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      .
      .
371 PSPIVVAVWRSFR ECRFVEDQGDVVFFK 398
   |. || |. || : | || .|| .|
450 PTPIPQAMWRVAKG CTAVEQRGDAVWVK 477

```

Figur 7: Polypeptidvergleich von Codierregionen der Δ-12-Desaturase aus *Mortierella alpina* (Ma_des12) obere Reihe mit der homologen Sequenz aus *Phaeodactylum tri-cornutum* Klon PT001072031R (Pt_des12.2) in der unteren Reihe.

```

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22 NSAKPAFERNYQLPEFTIKEIRECIPAHCFERSGLRGLCHVAIDLTWASL 71
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33 SSYNPLAKDSPELP..TKGQIKAVIPKECFORSAFWSTFYLMRDLAMAAA 80

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   .|:  : |      | | | | |      | : || ||. || || | |
81 FCYGT SQVLSTDL PQDATLILPWALGWGVYAFWMGTILTGPWVVAHECGH 130

      .           .           .           .           .
116 QSFSTSKTLNNTVGWILHSMLLVPYHSWRISHSKHHKATGHMTKDQVFVP 165
   .:| |. | |. ||. |. | | | | |. |. |. | | | | : | | : | |
131 GAYSDSQTFNDVVGFI VHQALLVPYFAWQYTHAKHRRRTNHLVDGESHVP 180

      .           .           .           .           .
166 KTRSQVGLPP..KENAAAVQEEDMSVHLDEEAPIVTLFWMVIQFLFGWP 213
   | | | | . |. | | |      : | | | | | | | | | | | |
181 STAKDNGLGPHNERN SFYAAWHEAMG....DGAFVAVFQVWS..HLFVWGP 224

      .           .           .           .           .
214 AYLI.MNASGQ..DYGRW.....TSHFHTYSPIFEPRNFFDIIISDLG 253
   || : ..|. | |      || | |. | : | : |
225 LYLAGLASTGKLAHEGWLEERNAIADHFRPSSPMFPAKIRAKIALSSAT 274

      .           .           .           .           .
254 VLAALGALIYASMQLSLLTVTKYYIVPYL FVNFWLVLITFLQHTDPKLP 303
   || | | |:| |. | | :| || || | | | | |. | | | | | | :| |
275 ELAVLAGLLYVGTQVGHL PVLLWYWGPTYFVNAWLVLVYTWLQHTDPSIPH 324

      .           .           .           .           .
304 YREGAWNFORGALCTVDRSFGKFLDHMFHGIVHTHVAHHLFSQMPFYHAE 353
   | | | | . :| | | |:| | :| | | | | | | | | | | | :| |. |. |
325 YGEGEWTWVKGALSTIDRDYGF.DFFHHTIGSTHVVHHLFHEMPWYNAG 373

      .           .           .           .           .
354 EATYHLKLLGE..YYVYDPSPIVVAVVWRSFRECRFVEDQGDVVFFK 398
   || .|. | | | | |. |. | | | | :| | | | | | :| |
374 IATQKVKEFLEPQGLYNDPTPWYKAMWRIARTCHYVESNEGVQYFK 420
    
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SEQUENZPROTOKOLL

<110> BASF Plant Science GmbH

<120> Verfahren zur Herstellung mehrfach ungesättigter
Fettsäuren, neue Biosynthesegene sowie neue pflanzliche
Expressionskonstrukte

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                                     55
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                                     75
                                     80
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                                     95
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His	Gly	Ala	Thr	Ser	Lys	Arg	Pro	Trp	Val	Asn	Asp	Met	Leu	Gly	Leu		
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Lys	Cys	Leu	Ser	Asn	Met	Ala	Ile	Trp	Ala	Ala	Ala	Cys	Ala	Leu	Val		
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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
 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 tgc 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

11

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

12

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	

14

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	

15

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				

<210> 8

<211> 525

<212> PRT

<213> Physcomitrella patens

<400> 8

Met Val Phe Ala Gly Gly Gly Leu Gln Gln Gly Ser Leu Glu Glu Asn
 1 5 10 15

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

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

<210> 9

<211> 873

<212> DNA

<213> Physcomitrella patens

<220>

<221> CDS

<222> (1)..(873)

<400> 9

atg gag gtc gtg gag aga ttc tac ggt gag ttg gat ggg aag gtc tcg 48

18

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1				5					10					15			
cag	ggc	gtg	aat	gca	ttg	ctg	ggc	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	ggc	ggt	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	ggt	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> 10
 <211> 290
 <212> PRT
 <213> *Physcomitrella patens*

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 1 5 10 15

Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
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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				
	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				
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Thr	Glu																		
	290																		

<210> 11
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 <212> DNA
 <213> Phaeodactylum tricornutum

<220>
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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

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		

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

tttcgcttaa aagatagttt tttctaccat ctgtgtagtc ggcacaaaaa aaaaaaaaaa 1522

aaaa 1526

<210> 12
 <211> 436
 <212> PRT
 <213> Phaeodactylum tricornutum

<400> 12
 Met Gly Lys Gly Gly Gln Arg Ala Val Ala Pro Lys Ser Ala Thr Ser
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 Ser Thr Gly Ser Ala Thr Leu Ser Gln Ser Lys Glu Gln Val Trp Thr
 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

23

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
 405 410 415

Gln Tyr Phe Lys Ser Met Glu Asn Val Pro Leu Thr Lys Asp Val Arg
 420 425 430

Asn Lys Ala Ala
 435

<210> 13
 <211> 3598
 <212> DNA
 <213> Unknown

<220>
 <223> Sequenz stellt eine pflanzliche Promotor-Terminator-Expressionskassette in Vektor pUC19 dar

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 cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120
 ttggcgggtg tcggggctgg cttactatg cggcatcaga gcagattgta ctgagagtgc 180
 accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcagggccc 240
 attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcggggc tcttogctat 300
 tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360
 tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cggcgcgccc agctcctcga 420
 gcaaatttac acattgccac taaacgtcta aacccttgta atttgtttt gttttactat 480
 gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540
 tttatgctaa cgtttgccaa cacttagcaa tttgcaagtt gattaattga ttctaaatta 600
 tttttgtctt ctaaatacat atactaatca actggaatg taaatatttg ctaatatttc 660
 tactatagga gaattaaagt gagtgaatat ggtaccacaa ggtttggaga ttaattggtt 720
 gcaatgctgc atggatggca tatacaccac acattcaata attcttgagg ataataatgg 780
 taccacacaa gatttgaggt gcatgaacgt cacgtggaca aaaggtttag taatttttca 840
 agacaacaat gttaccacac acaagttttg aggtgcatgc atggatgccc tgtggaaagt 900
 ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960
 ggaggatgca ataatagaaga aaactacaaa tttacatgca actagttatg catgtagtct 1020

atataatgag gattttgcaa tactttcatt catacacact cactaagttt tacacgatta 1080
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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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<210> 15

<211> 3584

<212> DNA

<213> Unknown

<220>

<223> Sequenz stellt eine pflanzliche
 Promotor-Terminator-Expressionskassette in Vektor
 pUC19 dar

<400> 15

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3584

<210> 16

<211> 4507

<212> DNA

<213> Unknown

<220>

<223> Sequenz stellt eine pflanzliche
Promotor-Terminator-Expressionskassette in Vektor
pUC19 dar

<400> 16

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Ala Ser Met Ser Leu Phe Ser Asp Phe Phe Ser Tyr Val Ser Ser Thr			
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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	
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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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Glu Leu Leu Lys Asp Phe Arg Glu Met Arg Ala Leu Phe Leu Arg Glu	
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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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Thr Ile Ser Ala Val Leu Ala Ser Ala Cys Met Met Ala Leu Cys Phe	
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Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His Asn Gln Val Phe	
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Glu Thr Arg Trp Leu Asn Glu Val Val Gly Tyr Val Ile Gly Asn Ala	

101

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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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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	
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Thr	Glu	Leu	Met	Ser	Gly	Met	Leu	Leu	Gly	Phe	Val	Phe	Val	Leu	Ser	
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Gln	Ile	Val	Ser	Thr	Arg	Asp	Ile	Lys	Gly	Asn	Ile	Phe	Asn	Asp	Trp	
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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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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	
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102

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 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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103

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Thr Glu Phe Glu Arg Glu Ile Lys Arg Glu Val Phe Lys Ile Val Arg	925	930	935	
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Arg Gly Lys Asp Phe Gly Thr Leu Gly Trp Phe Phe Arg Ala Phe Cys	940	945	950	
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Tyr Ile Ala Ile Phe Phe Tyr Leu Gln Tyr His Trp Val Thr Thr Gly	955	960	965	
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Thr Ser Trp Leu Leu Ala Val Ala Tyr Gly Ile Ser Gln Ala Met Ile	970	975	980	985
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Gly Met Asn Val Gln His Asp Ala Asn His Gly Ala Thr Ser Lys Arg	990	995	1000	
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Pro Trp Val Asn Asp Met Leu Gly Leu Gly Ala Asp Phe Ile Gly Gly	1005	1010	1015	
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Arg Phe Gln Ala Phe Phe Tyr Met Pro Val Leu Ala Gly Tyr Trp Leu	1070	1075	1080	
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104

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Gly Asn Ile Met Leu Met Gly Val Ala Glu Ser Leu Ala Leu Ala Val
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 Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
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 Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
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 Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
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 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
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 145 150 155 160
 Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
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 His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
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 Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
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 Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
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 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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Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
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His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
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Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
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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 5a3e55bccca1de9f33257b988d6198e8c5b068465	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	WO02057465A2.pdf	14084561 ec1bdfc103aed5c16dc776936af3c39c45145332	no	224
Warnings:					
Information:					
4	Non Patent Literature	AF532782.pdf	98457 33ea6fc016afbdc862aeb37ea2b04cf97168d591	no	2
Warnings:					
Information:					
5	Non Patent Literature	AY165023.pdf	97669 686b15e0aef4bd95a3ae9cf3c9684b4c16f4018f	no	2
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	no	6
			1d0042ea978bcebf3df03d1dd94bf828571c144		
Warnings:					
Information:					
8	Non Patent Literature	MOON_2001_45358.pdf	513318	no	10
			8c052d1617d2cd840a30c60db9180c0768f49653		
Warnings:					
Information:					
9	Non Patent Literature	Sayanova_1997_4211.pdf	376401	no	6
			2a2db428f431cf6360469fa1577d8d2546ebb9480		
Warnings:					
Information:					
10	Non Patent Literature	Shahidi_Bailey.pdf	59782	no	2
			84f236456fb9be76adcecc6123ed087833971df00		
Warnings:					
Information:					
11	Non Patent Literature	Sperling_2000_3801.pdf	1310295	no	11
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Warnings:					
Information:					
12	Non Patent Literature	Tonon_2003_440.pdf	424283	no	5
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Information:					
13	Non Patent Literature	CODEX.pdf	202023	no	15
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Warnings:					
Information:					

14	Non Patent Literature	Watts_1999_175.pdf	617310	no	8
			bf4f4bd7b4850011348054b5ee4cc78c55ff88ce		
Warnings:					
Information:					
15	Non Patent Literature	Girke.pdf	873321	no	10
			53292a6ee303c9a670efd8d2c9722515dcbd63c3		
Warnings:					
Information:					
16	Fee Worksheet (SB06)	fee-info.pdf	30879	no	2
			adcc33b258beb9ff8f1a98913cc1d6829defea94		
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.

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

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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
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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 <small>60f12519a780720f50db43678c01571c138f edde</small>	no	5

Warnings:

Information:	
Total Files Size (in bytes):	440924
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> 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.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> 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.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> 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.</p>	



UNITED STATES PATENT AND TRADEMARK OFFICE

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Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Includes details for application 15/256,914, inventor Petra Cirpus, and examiner ROBINSON, HOPE A.

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

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 negated 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

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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

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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,

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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

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				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
Sheet	1	of	2	Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
	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	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
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)				
	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
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*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.

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				Complete if Known		
				Application Number	15/256,914 – Conf.#4050	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				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	
Sheet	2	of	2			
<i>(Use as many sheets as necessary)</i>						

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.	
	CH	SAYANOVA, et al., "Expression of a Borage Desaturase cDNA Containing an N-Terminal Cytochrome b5 Domain Results in the Accumulation of High Levels of Δ6-Desaturated Fatty Acids in Transgenic Tobacco", Proc. Natl. Acad. Sci USA, 1997, Vol. 94, pp. 4211-4216.	
	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.	
	CL	WATTS, J. L., et al., "Isolation and Characterization of a Δ5-Fatty Acid Desaturase from Caenorhabditis elegans", Archives of Biochemistry and Biophysics, 1999, Vol. 362, No. 1, pp. 175-182.	
	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
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*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.

Search Notes 	Application/Control No. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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

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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 ω 3-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 ω 3-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 $\Delta 6$ -desaturase, a nucleic acid encoding a $\Delta 5$ -desaturase, a nucleic acid encoding a $\Delta 6$ -elongase, a nucleic acid encoding a $\omega 3$ -desaturase, a nucleic acid encoding a $\Delta 5$ -elongase, and a nucleic acid encoding a $\Delta 4$ -desaturase, wherein said nucleic acid encoding a $\Delta 5$ -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 $\Delta 5$ -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 $\Delta 5$ -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* 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^{Δ4,8,12,15,21})...” 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 ω 3-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 ω 3-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 ω 3-fatty acids. Indeed, the disclosed range is much less than the recited amount of polyunsaturated ω 3-fatty acids, and thus not even close to the polyunsaturated ω 3-fatty acids amount recited in amended claim 9. *Cirpus* does not teach or suggest the recited amount of polyunsaturated ω 3-fatty acids. Moreover, *Cirpus* does not teach the skilled person how to obtain a composition having the claimed content of polyunsaturated ω 3-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 ω 3-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

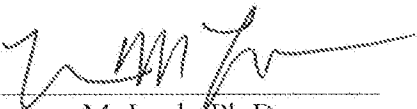
In view of the foregoing, Applicant(s) submit(s) that the pending claims are in condition for allowance, and respectfully request(s) reconsideration and timely allowance of the pending claims. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner 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).

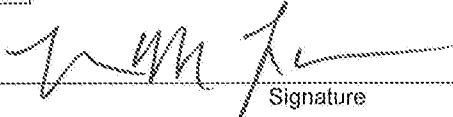
Dated: May 30, 2018

Respectfully submitted,

Customer Number: 123223

By 
Bronwen M. Loeb, Ph.D.
Registration No.: 43,516
DRINKER BIDDLE & REATH LLP
222 Delaware Avenue, Ste 1410
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Attorneys/Agents For Applicant

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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):				
	<u>Fee</u>	<u>Small Entity Fee</u>	<u>Micro Entity Fee</u>	
<input checked="" type="checkbox"/> One month (37 CFR 1.17(a)(1))	\$200	\$100	\$50	\$ 200.00
<input type="checkbox"/> Two months (37 CFR 1.17(a)(2))	\$600	\$300	\$150	\$ _____
<input type="checkbox"/> Three months (37 CFR 1.17(a)(3))	\$1,400	\$700	\$350	\$ _____
<input type="checkbox"/> Four months (37 CFR 1.17(a)(4))	\$2,200	\$1,100	\$550	\$ _____
<input type="checkbox"/> Five months (37 CFR 1.17(a)(5))	\$3,000	\$1,500	\$750	\$ _____
<input type="checkbox"/> Applicant asserts small entity status. See 37 CFR 1.27.				
<input type="checkbox"/> 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.				
<input type="checkbox"/> A check in the amount of the fee is enclosed.				
<input checked="" type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.				
<input type="checkbox"/> The Director has already been authorized to charge fees in this application to a Deposit Account.				
<input checked="" type="checkbox"/> The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number <u>50-0573</u>				
<input checked="" type="checkbox"/> 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				
<input type="checkbox"/> applicant.				
<input checked="" type="checkbox"/> attorney or agent of record. Registration number <u>43,516</u>				
<input type="checkbox"/> attorney or agent acting under 37 CFR 1.34. Registration number _____				
 Signature			<u>May 30, 2018</u> Date	
Bronwen M. Loeb, Ph.D. Typed or printed name			<u>202.230.5438</u> 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*.				
<input checked="" type="checkbox"/> * Total of <u>1</u> forms are submitted.				

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).

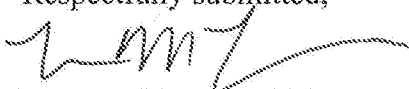
In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this

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,



Bronwen M. Loeb, Ph.D.
Registration No.: 43,516
DRINKER BIDDLE & REATH LLP
1500 K Street, N.W., Ste. 1100
Washington, District of Columbia 20005-1209
202.230.5438 (Phone)
302.351.6938 (Fax)
Attorneys/Agents For Applicant

Dated: May 30, 2018

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Substitute for form 1449/PTO			Complete if Known		
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)			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	
Sheet	1	of	1	Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)				
		20150361404		12-17-2015	BASF Plant Science GmbH	

FOREIGN PATENT DOCUMENTS							
Examiner Initials*	Cite No. ¹	Foreign Patent Document		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ³
		Country Code ⁴ Number ⁴ Kind Code ⁵ (if known)					

Examiner Signature		Date Considered	
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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 <i>Saccharomyces cerevisiae</i> . Fatty acid pattern of phospholipid classes and selective acyl turnover at sn-1 and sn-2 positions", <i>Yeast</i> , Vol. 10, 1994, pp. 1429-1437	
	CB	DIEDRICH, et al., "The natural occurrence of unusual fatty acids. Part 1. Odd numbered fatty acids", <i>Molecular Nutrition & Food Research</i> , Vol. 34, Issue 10, 1990, pp. 935-943	

Examiner Signature		Date Considered	
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*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)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Amendment.PDF	6497418	yes	13
			25817445225d8f0a4babc6f640c6aec56baab9428		

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	no	1
			795f72d0d3606038e1111aa7d7596a98478d81b0		

Warnings:

Information:

3		IDS_SB08.PDF	2020636	yes	3
			b3293c92e35fcbd1151d516384fce31b108d18fd		

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	yes	18
			32c01043df95b81da061a735153dd1bd4caf681		
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	no	2
			1806afd9be48bee01dee9a5387b674018563ca62		
Warnings:					
Information:					
Total Files Size (in bytes):			10240859		
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> 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.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> 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.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> 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.</p>					

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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/256,914	Filing Date 09/06/2016	<input type="checkbox"/> To be Mailed
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ENTITY: LARGE SMALL MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE (\$)	FEE (\$)
<input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
<input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
<input type="checkbox"/> 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 =	
<input type="checkbox"/> 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).			
<input type="checkbox"/> 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

	(Column 1)		(Column 2)	(Column 3)	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT	05/30/2018		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total (37 CFR 1.16(i))	*	23	Minus	** 23	= 0
	Independent (37 CFR 1.16(h))	*	1	Minus	*** 3	= 0
	<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))					
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

	(Column 1)		(Column 2)	(Column 3)	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT			CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total (37 CFR 1.16(i))	*		Minus	**	=
	Independent (37 CFR 1.16(h))	*		Minus	***	=
	<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))					
<input type="checkbox"/> 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.

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:

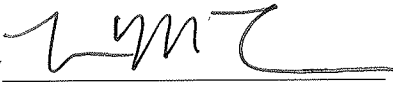
Applicant submits herewith the executed Declarations (37 CFR 1.63) For Utility or Design Application Using an Application Data Sheet (37 CFR 1.76) in the above-captioned application.

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

57672 VS02

PTO/AIA/01 (06-12)
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U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
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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: The attached application, or
 United States application or PCT international application number 15/266,914
filed on September 8, 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 identify 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: J. 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.

#85,083,082

576720802

PTO/AIA/01 (08-12)
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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: 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: 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.

57672 US02

PTO/AIA/D1 (08-12)
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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 The attached application, or
is directed to: 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 identify them. 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(e) 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: 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/D1 form for each additional inventor.

57672/US02

PTO/AIA/01 (08-12)

Approved for use through 01/31/2014. OMB 0881-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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DECLARATION (37 CFR 1.83) 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 The attached application, or is directed to:

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.

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 identify them. 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: Guchai WU

Date (Optional): April 19, 2017

Signature: Guchai 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.

#65,083,092

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**

As the below named inventor, I hereby declare that:

This declaration The attached application, or is directed to: 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 identify them. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2036 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-2036 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Tom WETJEN Date (Optional): 26. 09. 2016

Signature: *Tom Wetjen*

Note: An application data sheet (PTO/SB/15 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.

**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 The attached application, or
is directed to:

United States application or PCT international application number 15/256,914
filed on September 6, 2015

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:

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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.

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: The attached application, or

United States application or PCT international application number 15/255,914 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.

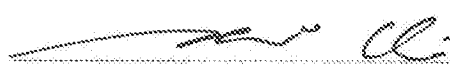
WARNING:

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: Xiao QIU

Date (Optional): July 3, 2018

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.

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
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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 <small>9314c5817c8e6f90a77ce47e09c59bf8d1803abd</small>	no	1

Warnings:

Information:					
2	Oath or Declaration filed	Declarations.pdf	5301376	no	7
			81b5d14255fb4de934f4abfd3c8721bee27720fa		
Warnings:					
Information:					
Total Files Size (in bytes):				5338775	
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> 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.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> 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.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> 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.</p>					



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

Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Includes application details for Petra Cirpus and examiner information for ROBINSON, HOPE A.

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

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. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 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. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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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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

Table with 5 columns: 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

Table with 7 columns: 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

<p>1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).</p> <p><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.</p> <p><input type="checkbox"/> "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.</p>	<p>2. For printing on the patent front page, list</p> <p>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively, _____ 1</p> <p>(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. _____ 2</p> <p>_____ 3</p>
---	---

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

<p>4a. The following fee(s) are submitted:</p> <p><input type="checkbox"/> Issue Fee</p> <p><input type="checkbox"/> Publication Fee (No small entity discount permitted)</p> <p><input type="checkbox"/> Advance Order - # of Copies _____</p>	<p>4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)</p> <p><input type="checkbox"/> A check is enclosed.</p> <p><input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.</p> <p><input type="checkbox"/> 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).</p>
---	--

5. **Change in Entity Status** (from status indicated above)

Applicant certifying micro entity status. See 37 CFR 1.29

Applicant asserting small entity status. See 37 CFR 1.27

Applicant changing to regular undiscouted fee status.

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.

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.

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 DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

Table with 5 columns: 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)

- | | |
|--|--|
| <ol style="list-style-type: none"> 1. <input type="checkbox"/> Notice of References Cited (PTO-892) 2. <input checked="" type="checkbox"/> Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____ 3. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit of Biological Material 4. <input checked="" type="checkbox"/> Interview Summary (PTO-413), Paper No./Mail Date <u>10/16/18</u>. | <ol style="list-style-type: none"> 5. <input checked="" type="checkbox"/> Examiner's Amendment/Comment 6. <input type="checkbox"/> Examiner's Statement of Reasons for Allowance 7. <input type="checkbox"/> 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.

Art Unit: 1652

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. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 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. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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:	
		17	
(Assistant Examiner)	(Date)	O.G. Print Claim(s)	O.G. Print Figure
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	10/17/18	1	NONE
(Primary Examiner)	(Date)		

Issue Classification 	Application/Control No. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 1652

<input checked="" type="checkbox"/> Claims renumbered in the same order as presented by applicant																<input type="checkbox"/> CPA		<input type="checkbox"/> T.D.		<input type="checkbox"/> R.1.47	
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original						

NONE		Total Claims Allowed:	
		17	
(Assistant Examiner)	(Date)	O.G. Print Claim(s)	O.G. Print Figure
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	10/17/18	1	NONE
(Primary Examiner)	(Date)		

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			<i>Complete if Known</i>		
INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>			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	
Sheet	1	of	1	Attorney Docket Number	074017-0013-01-US

U. S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)				
		20150361404		12-17-2015	BASF Plant Science GmbH	

FOREIGN PATENT DOCUMENTS							
Examiner Initials*	Cite No. ¹	Foreign Patent Document		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ³
		Country Code ⁴ Number ⁴ Kind Code ⁵ (if known)					

Examiner Signature		Date Considered	
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*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 <i>Saccharomyces cerevisiae</i> . Fatty acid pattern of phospholipid classes and selective acyl turnover at sn-1 and sn-2 positions", <i>Yeast</i> , Vol. 10, 1994, pp. 1429-1437	
	CB	DIEDRICH, et al., "The natural occurrence of unusual fatty acids. Part 1. Odd numbered fatty acids", <i>Molecular Nutrition & Food Research</i> , 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.

Search Notes 	Application/Control No. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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, transgenic 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

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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

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.

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. Enclosed

i. Amendment/Reply

ii. Affidavit(s)/ Declaration(s)

iii. Information Disclosure Statement (IDS)

iv. 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. 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. RCE fee required under 37 CFR 1.17(e)

ii. Extension of time fee (37 CFR 1.136 and 1.17)

iii. Other _____

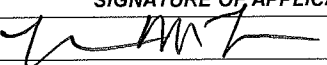
b. Check in the amount of \$ _____ enclosed

c. Payment by credit card (Form PTO-2038 enclosed)

d. 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

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 <h2 style="text-align: center;">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</h2> <p style="text-align: center;">(Use as many sheets as necessary)</p>				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
Sheet	1	of	2		

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	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
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)				

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	
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*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:

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	no	1
			17ce7283eeffc987e987c02b13ca50160d89443e		

Warnings:

This is not a USPTO supplied RCE SB30 form.

Information:

2		IDS.pdf	215533	yes	4
			e8ef024613081591ab4fdd1b07f848460d6e7d68		

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	no	29
			c942f871155f6a90c0a22c9e0b279360b186102f		

Warnings:

Information:

4	Non Patent Literature	074017-0013-01-US-541474_-_Thelen_2002_Metabolic_Engineering.pdf	230560	no	10
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Warnings:

Information:

5	Fee Worksheet (SB06)	fee-info.pdf	30602	no	2
			5c74dd418e475090cb45ef8ae98a008cb5729605		

Warnings:

Information:	
Total Files Size (in bytes):	813194
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> 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.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> 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.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> 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.</p>	



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Table with 7 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY.DOCKET.NO, TOT CLAIMS, IND CLAIMS. Row 1: 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



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

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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 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

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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.

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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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.

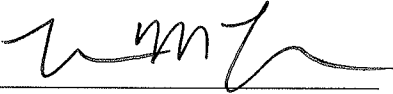
EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this

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,

Customer Number: 123223

By 
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
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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



Date Mailed: 11/21/2018

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Inventor(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

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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

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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
~~OILS, LIPIDS AND FATTY ACIDS PRODUCED IN TRANSGENIC BRASSICA PLANT~~

Preliminary Class

514

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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
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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	no	6
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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 ω 3-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 ω 3-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 $\Delta 6$ -desaturase, a nucleic acid encoding a $\Delta 5$ -desaturase, a nucleic acid encoding a $\Delta 6$ -elongase, a nucleic acid encoding a $\omega 3$ -desaturase, a nucleic acid encoding a $\Delta 5$ -elongase, and a nucleic acid encoding a $\Delta 4$ -desaturase, wherein said nucleic acid encoding a $\Delta 5$ -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 $\Delta 5$ -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 $\Delta 5$ -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 then need 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

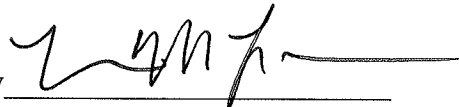
In view of the foregoing, Applicant(s) submit(s) that the pending claims are in condition for allowance, and respectfully request(s) reconsideration and timely allowance of the pending claims. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner 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,

Customer Number: 123223

By 
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202.230.5438 (Phone)
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Attorneys/Agents For Applicant



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Alexandria, Virginia 22313-1450
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Table with 5 columns: 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. 15/256,914	Applicant(s) CIRPUS ET AL.	
	Examiner HOPE ROBINSON	Art Unit 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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Table with 7 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY.DOCKET.NO, TOT CLAIMS, IND CLAIMS. Row 1: 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



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)

Petra Cirpus, Mannheim, GERMANY;
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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

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

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).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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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).

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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

Table with 5 columns: 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

Table with 7 columns: APPLN. TYPE, ENTITY STATUS, ISSUE FEE DUE, PUBLICATION FEE DUE, PREV. PAID ISSUE FEE, TOTAL FEE(S) DUE, DATE DUE

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), by mail or fax, or via EFS-Web.

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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)

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

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

<p>1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).</p> <p><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.</p> <p><input type="checkbox"/> "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.</p>	<p>2. For printing on the patent front page, list</p> <p>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively, _____ 1</p> <p>(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. _____ 2</p> <p>_____ 3</p>
---	---

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)

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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.

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Typed or printed name _____ Registration No. _____



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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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)

- | | |
|---|--|
| <ol style="list-style-type: none"> 1. <input type="checkbox"/> Notice of References Cited (PTO-892) 2. <input checked="" type="checkbox"/> Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____ 3. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit of Biological Material 4. <input type="checkbox"/> Interview Summary (PTO-413), Paper No./Mail Date _____. | <ol style="list-style-type: none"> 5. <input checked="" type="checkbox"/> Examiner's Amendment/Comment 6. <input type="checkbox"/> Examiner's Statement of Reasons for Allowance 7. <input type="checkbox"/> 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,

Art Unit: 1652

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.

Art Unit: 1652

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. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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:	
		23	
(Assistant Examiner)	(Date)	O.G. Print Claim(s)	O.G. Print Figure
/HOPE ROBINSON/ Primary Examiner.Art Unit 1652	12/7/18	1	NONE
(Primary Examiner)	(Date)		

ALL REFERENCES CONSIDERED EXCEPT WHERE SHOWN OTHERWISE 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)		<i>Complete if Known</i>	
		Application Number	15/256,914
		Filing Date	September 6, 2016
		First Named Inventor	Petra CIRPUS
		Art Unit	1652
		Examiner Name	H. A. Robinson
Sheet <u>1</u> of <u>2</u>	Attorney Docket Number	074017-0013-01 US-541474	


U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	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
		Country Code ³ Number ⁴ Kind Code ⁵ (if known)				

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
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*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. 15256914	Applicant(s)/Patent Under Reexamination CIRPUS ET AL.
	Examiner HOPE ROBINSON	Art Unit 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, transgenic 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

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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



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Includes fields for EXAMINER (ROBINSON, HOPE A), ART UNIT (1652), PAPER NUMBER, NOTIFICATION DATE (12/20/2018), and DELIVERY MODE (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



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

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

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) _____.

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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.

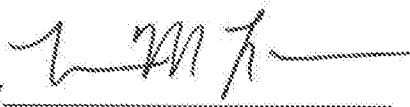
A copy of the Notice to File Corrected Application Papers is attached.

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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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".*

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Application No. 15256914

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Specification document coded dated , page(s) .

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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: .

SUBSTITUTE SPECIFICATION-CLEAN

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

10 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 $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity, where the nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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 $\omega 3$ -desaturase and/or of a $\Delta 4$ -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-

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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.

5 The invention furthermore relates to recombinant nucleic acid molecules comprising at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity and which is modified by comparison with the nucleic acid sequence in the organism from which the
10 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
15 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-
20 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
25 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 Prokaryotes*. 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
30 resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This

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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.

5 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).

10 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 Engineering*, Ed.: JK Setlow, 18:111-13; Gerhardt (1992) *Prog. Lipid R.* 31:397-417; Gühnemann-Schäfer & Kindl
15 (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.

20 Depending on the desaturation pattern, two large classes of polyunsaturated fatty acids, the $\omega 6$ and the $\omega 3$ 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).

25 The fatty acid linoleic acid ($18:2^{\Delta 9,12}$) acts as starting material for the $\omega 6$ metabolic pathway, while the $\omega 3$ pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed from linoleic acid by the activity of an $\omega 3$ -desaturase (Tocher et al. (1998) *Prog. Lipid Res.* 37: 73-117; Domergue et al. (2002) *Eur. J. Biochem.* 269: 4105-4113).

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Mammals, and thus also humans, have no corresponding desaturase activity ($\Delta 12$ - and $\omega 3$ -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 $\omega 6$ -fatty acid and the two
5 $\omega 3$ -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
10 (KCS, hereinbelow referred to as elongase). This is followed by a reduction step (ketoacyl-CoA reductase, KCR), a dehydration 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
15 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 $\omega 3$ -fatty acids are supposed to have a positive effect on the
20 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 $\omega 3$ -fatty acids to the food (Shimikawa (2001) World Rev. Nutr. Diet. 88: 100-108).

$\omega 3$ -fatty acids also have a positive effect on inflammatory, specifically on chronically
25 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. $\omega 6$ -fatty acids such as arachidonic acid tend to have a negative effect in connection with these rheumatological diseases.

$\omega 3$ - and $\omega 6$ -fatty acids are precursors of tissue hormones, known as eicosanoids, such as the
30 prostaglandins, which are derived from dihomo- γ -linolenic acid, arachidonic acid and

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eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG₂ series) which are formed from the ω₆-fatty acids, generally promote inflammatory reactions, while eicosanoids (known as the PG₃ series) from ω₃-fatty acids have little or no proinflammatory effect.

- 5 Polyunsaturated long-chain ω₃-fatty acids such as eicosapentaenoic acid (= EPA, C20:5^{Δ5,8,11,14,17}) or docosahexaenoic acid (= DHA, C22:6^{Δ4,7,10,13,16,19}) 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
10 (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^{Δ4,7,10,13,16,19}) or eicosapentaenoic acid (= EPA, C20:5^{Δ5,8,11,14,17}) are added to infant formula to improve the
15 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 *Cryptocodinium* or *Phaeodactylum* and others, being obtained, as a rule, in the
20 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^{Δ5,8,11,14}), dihomo-γ-linolenic acid (DHGL, C20:3^{Δ8,11,14}) or docosapentaenoic acid (DPA, C22:5^{Δ7,10,13,16,19}) are, however, not synthesized in oil crops such
25 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
30 acids or triglycerides for the production of oils in various organisms with a modified content of

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unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describe a Δ^9 -desaturase. WO 93/11245 claims a Δ^{15} -desaturase and WO 94/11516 a Δ^{12} -desaturase. Further desaturases 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).

5

10 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. Δ^6 -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.

15 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.

20 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 (C₂₈-C₃₂). The synthesis of arachidonic acid and EPA is described, for example, in WO

25 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 tricorutum*, *Porphiridium species*, *Thraustochytrium species*, *Schizochytrium*

30 species or *Cryptocodinium species*, ciliates such as *Stylonychia* or *Colpidium*, fungi such as

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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
5 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,
10 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
15 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
20 encode enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. These genes encode for example $\Delta 6$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases or $\Delta 4$ -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 $\Delta 6$ -desaturase genes from the moss *Physcomitrella patens*
25 and $\Delta 6$ -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
30 which are present in the plants. Thus, the ARA content in the plants described in DE-A-102 19

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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 $\Delta 5$ -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 $\Delta 6$ -desaturase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 6$ -elongase activity; at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -desaturase activity; and at least one nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -elongase activity,

where the nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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 $\Delta 4$ -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 $\Delta 6$ -desaturase activity,

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a polypeptide having a $\Delta 6$ -elongase activity, a polypeptide having a $\Delta 5$ -desaturase activity and a polypeptide having a $\Delta 5$ -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
5 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 $\Delta 5$ -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
10 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
15 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
20 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
25 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

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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.jp/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 $\Delta 5$ -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 $\Delta 5$ -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 $\Delta 5$ -elongase and whose amino

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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 $\Delta 6$ -desaturase activity is preferably selected from the group consisting of:

- 5 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 NO: 2,
- 10 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%,
15 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:
20 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
25 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 $\Delta 6$ -elongase activity is preferably selected from the group consisting of:

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- 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:
5 172,
- 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
10 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,
15 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 $\Delta 6$ -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.

- 20 The nucleic acid sequence which codes for a polypeptide having a $\Delta 5$ -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,
25 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,

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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

5 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 Δ 4-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.

25 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

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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 $\Delta 4$ -desaturase activity is preferably selected from the group consisting of:

- 5 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,
- 10 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%,
15 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.

- 20 The $\Delta 4$ -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
25 to the nucleic acid sequences which code for polypeptides having a $\Delta 6$ -desaturase activity, a $\Delta 6$ -elongase activity, a $\Delta 5$ -desaturase activity and a $\Delta 5$ -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 $\Delta 4$ -desaturase activity.

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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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and the $\Delta 5$ -elongase, and if appropriate the $\omega 3$ -desaturase and/or the $\Delta 4$ -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, bulbils 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

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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.

5 “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. 15 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 20 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.

25 “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. 30 4: 2723-2729).

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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
5 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
10 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
15 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
20 (*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),
25 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 $\Delta 5$ -elongase and which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates
30 by being adapted to the codon usage in one or more plant species, preferably the nucleic acid

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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 $\Delta 5$ -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 $\Delta 4$ -desaturase activity are required. A nucleic acid sequence which codes for a polypeptide having the activity of a $\Delta 4$ -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.

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In a further particularly preferred embodiment of the present invention, the nucleic acid sequences used, especially the nucleic acid sequence which codes for a $\Delta 5$ -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).

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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
5 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
10 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-
15 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
20 fatty acids, particularly preferably C₂₀ or C₂₂ 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,
25 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

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(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
5 phosphatidylinositol.

The fatty acid esters with polyunsaturated C₁₈-, C₂₀- and/or C₂₂-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,
10 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,
15 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
20 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,
25 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,

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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 (11-octadecen-9-ynoic acid), 6,9-octadecenynonic

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acid, pyrulic acid (110-heptadecen-8-ynonic acid), crepenynic acid (9-octadecen-12-ynonic acid) 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diyonic 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), puniolic 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^{Δ^{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,

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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*, *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*.

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 annuus* (sunflower), *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* (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*, *Arabidopsis*, 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*

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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 sativa* (hemp), *Convolvulaceae* such as the genera *Ipomoea*, *Convolvulus*, e.g. the genera and species *Ipomoea batatas*, *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 *Cucurbita*, 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 *Ricinus communis* (castor oil plant), *Fabaceae* such as the genera *Pisum*, *Albizia*, *Cathormion*, *Feuillea*, *Inga*, *Pithecolobium*, *Acacia*, *Mimosa*, *Medicago*, *Glycine*, *Dolichos*, *Phaseolus*, *Soja*, e.g. the genera and species *Pisum sativum*, *Pisum arvense*, *Pisum humile* (pea), *Albizia berteriana*, *Albizia julibrissin*, *Albizia lebeck*, *Acacia berteriana*, *Acacia littoralis*, *Albizia berteriana*, *Albizia berteriana*, *Cathormion berteriana*, *Feuillea berteriana*, *Inga fragrans*, *Pithecellobium berterianum*, *Pithecellobium fragrans*, *Pithecolobium berterianum*, *Pseudalbizia berteriana*, *Acacia julibrissin*, *Acacia nemu*, *Albizia nemu*, *Feuillea julibrissin*, *Mimosa julibrissin*, *Mimosa speciosa*, *Sericanrda julibrissin*, *Acacia lebeck*, *Acacia macrophylla*, *Albizia lebbek*, *Feuillea lebeck*, *Mimosa lebeck*, *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*

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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), *Lythraeae* 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*

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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*
5 *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*
10 *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*,
15 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*
20 *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
25 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,
30 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

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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 viciifolia*), Egyptian clover (*Trifolium alexandrinum*) and Persian clover (*Trifolium resupinatum*). Preferred food plants are for instance lettuce species such as *Lactuca sativa*, *Lactuca crispera*, *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 $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity, advantageously in combination with nucleic acid sequences which code for polypeptides having $\omega 3$ -desaturase and/or $\Delta 4$ -desaturase activity, and further nucleic acid sequences which code for polypeptides of fatty acid or lipid metabolism, such as further polypeptides having $\Delta 5$ -, $\Delta 6$ -, $\Delta 8$ -, $\Delta 12$ -desaturase or $\Delta 5$ -, $\Delta 6$ - or $\Delta 9$ -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,

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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 Δ^6 -elongase, Δ^6 -desaturase, Δ^5 -desaturase and/or Δ^6 -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 Δ^{12} -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 Δ^{12} -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

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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*, *Pycnococcus*, *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 anylifera*, *Pyramimonas disomata*, *Pyramimonas obovata*, *Pyramimonas orientalis*, *Pyramimonas parkae*, *Pyramimonas spinefera*, *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* or *Tetraselmis* sp. or algae from the family *Euglenaceae* 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

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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*,
5 *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*,
10 *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 *Cryptothecodinium*.

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
15 for a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase and, if appropriate, nucleic acid sequences which code for an $\omega 3$ -desaturase and/or a $\Delta 4$ -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
20 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
25 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
30 example, a nucleic acid sequence, an expression cassette (= gene construct) or a vector

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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

- 5 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 15 500 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -elongase activity, advantageously in combination with nucleic acid sequences which encode proteins having $\omega 3$ -desaturase and/or $\Delta 4$ -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.

25 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 30 with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences

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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.

- 5 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*,
10 *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*.
- 15 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
20 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
25 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.

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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.

5 “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, cotyledons, 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
10 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
15 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
20 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
25 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 C₂₀ and/or C₂₂ fatty acid
30 molecules having at least four double bonds in the fatty acid molecule, preferably five or six

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double bonds. These C₂₀ and/or C₂₂ 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.

5 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 15 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-dienonic acid), vernonic acid (9,10-epoxyoctadec-12-enonic acid), tarinic acid (6-octadecynonic acid), 6-nonadecynonic acid, santalbic acid (11-octadecen-9-ynoic acid), 6,9-octadecynonic acid, pyrulic acid (11-o-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), puniolic 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 30

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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^{Δ3,8,12,15,18,21}).

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.

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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, 5 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 10 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, H₂SO₄. 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 $\Delta 6$ - 15 desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity and optionally nucleic acid sequences which encode polypeptides having $\omega 3$ -desaturase and/or $\Delta 4$ -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), 20 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 C₁₆-, C₁₈- or C₂₀- fatty acids. Preferably, the fatty acids converted in the process as substrates are converted in the 25 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 C₁₆-fatty acids and/or polyunsaturated C₁₈-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 30 elongation cycle, this enzyme activity leads either starting from C₁₆-fatty acids to C₁₈-fatty acids

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or starting from C₁₈-fatty acids to C₂₀-fatty acids, and after two elongation cycles starting from C₁₆-fatty acids leads to C₂₀-fatty acids. The activity of the desaturases or elongases used in the process according to the invention preferably leads to C₂₀- and/or C₂₂-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 C₂₂-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 C₁₈-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.

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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
5 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
10 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 $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, and/or $\omega 3$ -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
15 $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, and/or $\omega 3$ -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
20 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 amplicon 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 protocol (for example Qiagen). An
25 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
30 which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule,

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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 amplicon 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.

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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.

5 Owing to the introduction of a $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -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
10 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase genes which are
15 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,
20 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and/or $\Delta 5$ -elongase activity and, if appropriate, a $\Delta 4$ -desaturase
25 and/or $\omega 3$ -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
30 least about 60% and preferably at least about 70%, 80% or 90%, and particularly preferably at

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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.

5 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
10 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
15 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 ω 3-desaturase, Δ 6-desaturase, Δ 6-elongase, Δ 5-elongase, Δ 4-desaturase and/or Δ 5-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
20 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
25 molecules across membranes.

Nucleic acids which can be advantageously used in the process are derived from bacteria, fungi, diatoms, animals such as *Caenorhabditis* 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*,
30 *Mortierella*, *Borago*, *Phaeodactylum*, *Crypthecodinium*, specifically from the genera and species

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Pytium irregulare, *Oncorhynchus mykiss*, *Xenopus laevis*, *Ciona intestinalis*, *Thalassiosira pseudonona*, *Mantoniella squamata*, *Ostreococcus* sp., *Ostreococcus tauri*, *Euglena gracilis*, *Physcomitrella patens*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp.,
5 *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 $\Delta 12$ -desaturase, $\Delta 9$ -elongase or $\Delta 8$ -desaturase. The nucleic acid sequences used in the
10 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 $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 4$ -desaturase are functionally linked to one or more regulatory signals to increase the gene expression. These
15 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
20 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
25 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
30 sequences can also be inserted at the 3' end of the DNA sequences, such as further regulatory

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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 constructs 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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase or $\Delta 5$ -elongase and if appropriate the $\omega 3$ -desaturase or $\Delta 4$ -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.

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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, $\Delta 8$ -desaturase, $\Delta 9$ -desaturase, $\Delta 12$ -desaturase and/or $\Delta 9$ -elongase.

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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

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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 $\Delta 6$ -desaturates, $\Delta 6$ -elongase, $\Delta 5$ -desaturate and/or $\Delta 5$ -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;

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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, λ gt11 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 pYeDesaturase1 (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

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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

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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,

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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 $\Delta 6$ -desaturase, $\Delta 6$ -elongase or $\Delta 5$ -desaturase and, if appropriate, the $\omega 3$ -desaturase or $\Delta 4$ -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

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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 $\Delta 5$ -elongase, $\omega 3$ -desaturase, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 4$ -desaturase or $\Delta 5$ -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,

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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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase and/or Δ 6-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

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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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase and/or Δ 6-elongase which is used in the process and

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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 ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-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 ω 3-desaturase-, Δ 6-desaturase-, Δ 5-desaturase-, Δ 5-elongase-, Δ 4-desaturase- or Δ 6-elongase-encoding sequence, e.g. by saturation mutagenesis, and the resulting mutants can be screened for the ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-elongase activity described herein in order to identify mutants which have retained the ω 3-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase, Δ 4-desaturase or Δ 6-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

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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	<i>Ostreococcus tauri</i>
2	65	<i>Ostreococcus tauri</i>
3	1	<i>Phytium irregulare</i>
4	2	<i>Phytium irregulare</i>
5	171	<i>Traustochytrium sp.</i>
6	172	<i>Traustochytrium sp.</i>
7	51	<i>Thraustochytrium ssp.</i>
8	52	<i>Thraustochytrium ssp.</i>
9	193	<i>Phytophthora infestans</i>
10	194	<i>Phytophthora infestans</i>
11	77	<i>Traustochytrium sp.</i>
12	78	<i>Traustochytrium sp.</i>
13	109	<i>Ostreococcus tauri</i>
n.a.	110	<i>Ostreococcus tauri</i>
14	122	<i>Ostreococcus tauri</i>
n.a.	123	<i>Ostreococcus tauri</i>
15	143	<i>Ostreococcus tauri</i>

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16	144	<i>Ostreococcus tauri</i>
17	161	<i>Cauliflower mosaic virus</i>
18	162	<i>Cauliflower mosaic virus</i>
19	163	<i>Thalassiosira pseudonana</i>
20	164	<i>Thalassiosira pseudonana</i>

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 $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity. These are the following sequences:

Gene name	SEQ ID	Amino acids
OtELO1.1, ($\Delta 6$ -Elongase)	SEQ ID NO: 143	292
OtELO2.1, ($\Delta 5$ -Elongase)	SEQ ID NO: 109	300

OtElo2.1 shows greatest similarity to an elongase from *Danio rerio* (GenBank AAN77156;

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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:

- 5 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
- 10 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*

- 15 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 (Δ 6-elongase) and SEQ ID NO: 109 (coding for a protein identified by SEQ ID NO: 110) (Δ 5-elongase) were adapted to the codon usage in oilseed rape, flax and soybean. For this purpose, the amino acid sequence of the Δ 6-elongase and of the Δ 5-elongase (SEQ ID NO: 144
- 20 for the Δ 6-elongase; SEQ ID NO: 65 for the Δ 5-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 (Δ 5-elongase) and SEQ ID NO: 122
- 25 (coding for a protein identified by SEQ ID NO: 123) (Δ 6-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 Δ 6-

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elongase sequence) and pOTE2.2 (comprising the Δ 5-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, (Δ 6-elongase)	SEQ ID NO: 143	292
pOTE1.2, (Δ 6-elongase)	SEQ ID NO: 122	292, codon-optimized
pOTE2.1, (Δ 5-elongase)	SEQ ID NO: 109	300
pOTE2.2, (Δ 5-elongase)	SEQ ID NO: 64	300, codon-optimized

The *Saccharomyces cerevisiae* strain 334 was transformed by electroporation (1500 V) with the
5 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 Δ 6-elongase and Δ 5-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
10 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 OD₆₀₀ of 0.05.
15 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
20 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

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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 NaHCO₃, 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 NaHCO₃, pH 8.0 and with 2 ml of distilled water. The PE phases were then dried with Na₂SO₄, 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 $\Delta 5$ -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

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The following general conditions 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:

10 Cn11 C 5': gaattcggegcgccgagctcctcgagcaacgggtccggcggtatagagttgggtaattcga (SEQ ID NO: 200)

Cn11 C 3': cccgggatcgatgccggcagatctccaccatttttggtggtgat (SEQ ID NO: 201)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

15 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

20 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

25

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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.

- 5 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.

- 10 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)

- 15 Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

- 20 0.50 µl of Advantage polymerase (Clontech)

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

- 25 Elongation temperature: 2 min 72°C

Number of cycles: 35

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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-Cn11-C_OCS was verified by sequencing.

10 In the next step, the Cn11-B promoter was amplified by PCR by means of the following primers:

Cn11-B 5': aggcctcaacgggtccggcggtatag (SEQ ID NO: 204)

Cn11-B 3': cccgggggtaacgctagegggcccgatatcggatcccatttttggtggtgattggttct (SEQ ID NO: 205)

Composition of the PCR mix (50 µl):

15 5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

20

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

25 Number of cycles: 35

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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
5 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-Cn11-C_Cn11B_OCS was verified by sequencing.

In a further step, the OCS terminator for Cn11B was inserted. To this end, the PCR was carried
10 out using the following primers:

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

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

Composition of the PCR mix (50 µl):

- 15 5.00 µl template cDNA
- 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂
- 5.00 µl of 2mM dNTP
- 1.25 µl of each primer (10 pmol/µl)
- 0.50 µl of Advantage polymerase (Clontech)

20

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

25 Number of cycles: 35

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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:

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

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

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

15 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

20 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

25

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The PCR product was incubated for 2 hours at 37°C with the restriction enzyme *StuI*. The vector pUC19-Cn11-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-Cn11C_Cn11B_Cn11A_OCS2 was verified by sequencing.

In a further step, the OCS terminator for Cn11A was inserted. To this end, the PCR was carried out with the following primers:

- 10 OCS2 5': ggccctcctgctttaatgagatatgcca (SEQ ID NO: 210)
OCS2 3': aagcttggegcgcccagctcgtcgacggacaatcagtaaattgaacggaga (SEQ ID NO: 211)

Composition of the PCR mix (50 µl):

- 5.00 µl template cDNA
15 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂
5.00 µl of 2mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl of Advantage polymerase (Clontech)

20 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

25

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- 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
- 5 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.
- 10 In the next step, the plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the $\Delta 6$ -, $\Delta 5$ -desaturase and $\Delta 6$ -elongase. To this end, the *Phytium irregulare* $\Delta 6$ -desaturase (WO02/26946) was amplified using the following PCR primers:

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

- 15 D6Des(Pir) 3': ccatggccccgggttacatcgctgggaactcggat (SEQ ID NO: 213)

Composition of the PCR mix (50 μ l):

- 5.00 μ l template cDNA
- 5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂
- 20 5.00 μ l of 2mM dNTP
- 1.25 μ l of each primer (10 pmol/ μ l)
- 0.50 μ l of Advantage polymerase (Clontech)

PCR reaction conditions:

- 25 Annealing temperature: 1 min 55°C
- Denaturation temperature: 1 min 94°C

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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/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/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. $\Delta 5$ -desaturase (WO02/26946). To this end, the *Thraustochytrium* ssp. $\Delta 5$ -desaturase was amplified using the following PCR primers:

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

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

Composition of the PCR mix (50 μ l):

5.00 μ l template cDNA

5.00 μ l 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 μ l of 2mM dNTP

1.25 μ l of each primer (10 pmol/ μ l)

0.50 μ l of Advantage polymerase (Clontech)

25

PCR reaction conditions:

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Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

Number of cycles: 35

5

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 *EcoRV*. 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
10 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.

15 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': gggccgcacatggaggtcgtggagagattctacggtg (SEQ ID NO: 216)

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

20

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

25 1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

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PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

5 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-
10 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
15 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
20 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
25 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:

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Cn11_OCS 5': gtcgatcaacgggtccggcggtatagagttg (SEQ ID NO: 218)

Cn11_OCS 3': gtcgatcggacaatcagtaaattgaacggaga (SEQ ID NO: 219)

Composition of the PCR mix (50 µl):

- 5 5.00 µl template cDNA
- 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂
- 5.00 µl of 2mM dNTP
- 1.25 µl of each primer (10 pmol/µl)
- 0.50 µl of Advantage polymerase (Clontech)

10

PCR reaction conditions:

Annealing temperature: 1 min 55°C

Denaturation temperature: 1 min 94°C

Elongation temperature: 2 min 72°C

- 15 Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzyme Sall. The vector pUC19 was incubated for 2 hours at 37°C with the restriction enzyme Sall. 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-Cn11_OCS was verified by sequencing.

20 In a further step, the *Calendula officinalis* Δ12-desaturase gene (WO01/85968) was cloned into pUC19-Cn11_OCS. To this end, d12Des(Co) was amplified with the following primers:

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

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

Composition of the PCR mix (50 µl):

5.00 µl template cDNA

5 5.00 µl 10x buffer (Advantage polymerase) + 25mM MgCl₂

5.00 µl of 2mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl of Advantage polymerase (Clontech)

10 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

15

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.

20 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
25 pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) were incubated for 2 hours at 37°C with the restriction enzyme Sall. Thereafter, the vector fragment and the cleaved vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was

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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.

5 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 *AscI*. 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
10 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
15 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
20 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
25 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
30 treated vector pSUN-GW. The plasmid obtained in this way pSUN-4G was used for further gene

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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
5 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
10 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.

15 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
20 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
25 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,

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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: 5 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 10 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, 15 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 20 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 25 metabolites 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.

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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).

5 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).

10 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
15 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.
20 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
25 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
30 with corresponding FAME standards (Sigma). The identity and position of the double bond can

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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 $\Delta 5$ -elongase (as depicted in SEQ ID NO: 64) from *Ostreococcus tauri* for constructs for constitutive expression

5 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 Sall/XbaI
10 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 $\Delta 6$ -desaturase (SEQ ID NO: 1), of the $\Delta 5$ -desaturase (SEQ ID NO: 51) and $\Delta 6$ -elongase (SEQ ID NO: 171) sequences were inserted via the EcoRV
15 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 Sall, the plasmid pUC-D6 was digested with Sall/XhoI, and the correct fragments were ligated. The resulting plasmid pGPTV-D6 was then digested with Sall, the plasmid pUC-D5 was digested with Sall/XhoI, and the correct fragments were ligated.
20 The resulting plasmid pGPTV-D6-D5 was then digested once more with Sall, the plasmid pUC-E6(Tc) with Sall/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
25 prepare the binary vector pGPTV-35S_D6D5E6(Tp).

In a further procedure, the open reading frame of $\omega 3$ Des (SEQ ID NO: 193) was cloned into pUC19-35S. The resulting plasmid pUC- $\omega 3$ Pi was transferred via Sall/XhoI into the binary vectors pGPTV-D6D5E6(Tc) and pGPTV-D6D5E6(Tp). The resulting vectors pGPTV-D6D5E6(Tc) $\omega 3$ Pi and pGPTV-D6D5E6(Tp) $\omega 3$ Pi were employed for the plant transformation.

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In a further procedure, the open reading frame of the optimized $\Delta 5$ -elongase from *Ostreococcus tauri* (SEQ ID NO: 64) and the open reading frame of the $\Delta 4$ -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
5 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).

10 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
15 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 cm²) were incubated
20 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,
25 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-
30 environment cabinet or in the greenhouse, allowed to flower, mature seeds were harvested and

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analyzed for elongase expression such as $\Delta 6$ -elongase activity or for $\Delta 5$ - or $\Delta 6$ -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

- 5 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) ω 3PiE5D4 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
10 *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 cm²) 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
15 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 μ M 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.

- 20 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 $\Delta 6$ -elongase activity or $\Delta 5$ - or $\Delta 6$ -desaturase activity. Lines with increased contents of polyunsaturated C20 and C22 fatty acids
25 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) ω 3PiE5D4 was

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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
5 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
10 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
15 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
20 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
25 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*

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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
5 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
10 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.

15 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,
20 resuspended in aqueous buffer by brief ultrasound treatment (1 M Tris pH 8; 2.2% CaCl₂ (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
25 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: $sn-1 + sn-3 = (TAG \times 3 - MAG)/2$.

30 This position analysis of the triacylglycerides revealed in this case that EPA and DHA are

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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

- 5 Fractionated and extracted phosphatidylglycol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were dried under N₂ and resuspended in 0.5 ml of borate buffer (0.5M, pH 7.5, containing 0.4 mM CaCl₂). 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
- 10 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.
- 15 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
- 20 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.

- 25 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 CaCl₂), 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

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digested galactolipids were in a small volume of chloroform:methanol (2:1 v/v) and developed twice on a pre-coated 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 <i>Ostreococcus tauri</i> 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

SUBSTITUTE SPECIFICATION-CLEAN

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 <i>Orychophragmus violaceus</i>											
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 <i>Arabidopsis thaliana</i>											
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	0,45	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	0,12	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	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	9,72	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	γ 18:3	α 18:3	18:4	20:0		
B1223	PUFA184_MKP71_581A	4,4	3,0	22,5	16,9	27,0	4,9	3,2	0,6		
B1223	PUFA184_MKP71_581A	4,7	3,9	17,9	10,6	29,5	4,2	4,0	0,9		
B1223	PUFA184_MKP71_581A	4,4	3,0	18,9	13,8	30,5	4,1	3,2	0,7		
B1223	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
Attorney Docket Number:	074017-0013-01-US
Receipt Date:	16-JAN-2019
Filing Date:	06-SEP-2016
Time Stamp:	11:09:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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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

Multipart Description/PDF files in .zip description			
Document Description	Start	End	
Applicant Response to Pre-Exam Formalities Notice	1	1	
Specification	2	2	
Applicant Arguments/Remarks Made in an Amendment	3	5	

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2	Specification	SubSpecClean_1_88.pdf	2065640	no	88
			6ca984fa7753e4318aea72be5cf8f67a3f1b1723		

Warnings:

Information:

Total Files Size (in bytes):		4204170
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New Applications Under 35 U.S.C. 111

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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.

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Commissioner for Patents
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Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

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222 Delaware Avenue, Ste 1410
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(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-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

<p>1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)</p> <p><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.</p> <p><input type="checkbox"/> "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.</p>	<p>2. For printing on the patent front page, list</p> <p>1. Drinker Biddle & Reath LLP</p> <p>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,</p> <p>2.</p> <p>(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.</p> <p>3.</p>
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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)

BASF Plant Science GmbH

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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- 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:				
Pages:				
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
Deposit Account	
Authorized User	

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1	Issue Fee Payment (PTO-85B)	IssueFeeTransmittal.pdf	17475	no	1
			95562e22525p92e8332d8bb9e3758f1a16c340ac		

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30565	no	2
			5c179f7f48285334da8317ea87cd3dd8eeea47cb		

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New Applications Under 35 U.S.C. 111

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Table with 5 columns: 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.	Applicant(s)
	15/256,914	
	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

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Substitute for form 1449/PTO				<i>Complete if Known</i>	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				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
Sheet	1	of	1	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 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
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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 <i>Saccharomyces cerevisiae</i> . Fatty acid pattern of phospholipid classes and selective acyl turnover at sn-1 and sn-2 positions", <i>Yeast</i> , Vol. 10, 1994, pp. 1429-1437	
	CB	DIEDRICH, et al., "The natural occurrence of unusual fatty acids. Part 1. Odd numbered fatty acids", <i>Molecular Nutrition & Food Research</i> , Vol. 34, Issue 10, 1990, pp. 935-943	

Examiner Signature	/HOPE A ROBINSON/	Date Considered	10/18/2018
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¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

Change(s) applied to document, N.B.H./ 1/5/2019



APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/256,914	05/28/2019	10301638	074017-0013-01-US	4050

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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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