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(54) METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS IN TRANSGENIC PLANTS

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(58) Field of Classification Search None

See application file for complete search history.

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(57)ABSTRACT

The present invention relates 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 ω 3-desaturase, Δ 12-desaturase, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and/or Δ 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.

25 Claims, 33 Drawing Sheets

CSIRO Exhibit 1013

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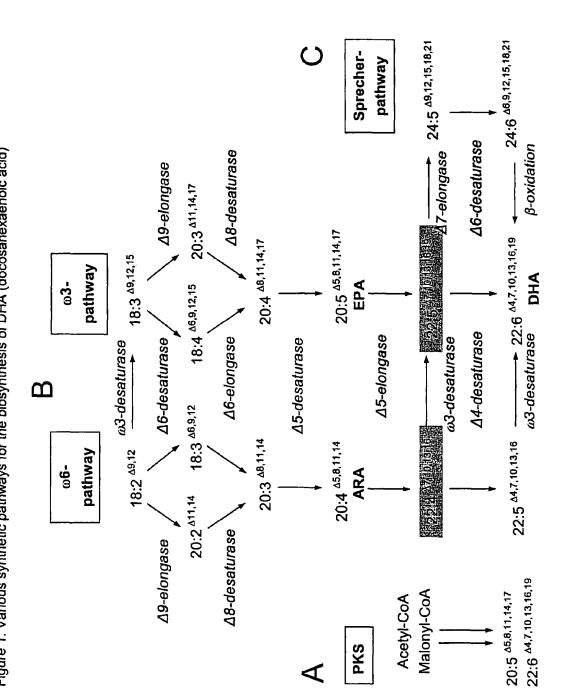




Figure 2: Substrate specificity of the ∆5-elongase (SEQ ID NO: 53) with regard to different fatty acids

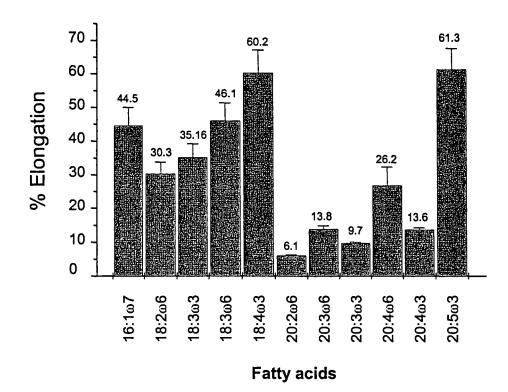
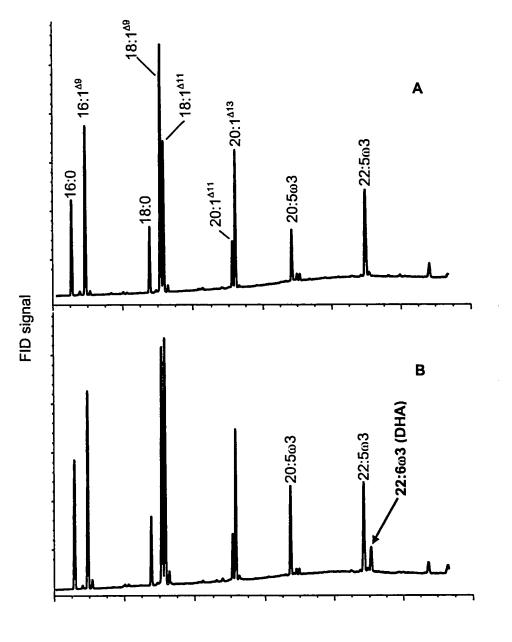
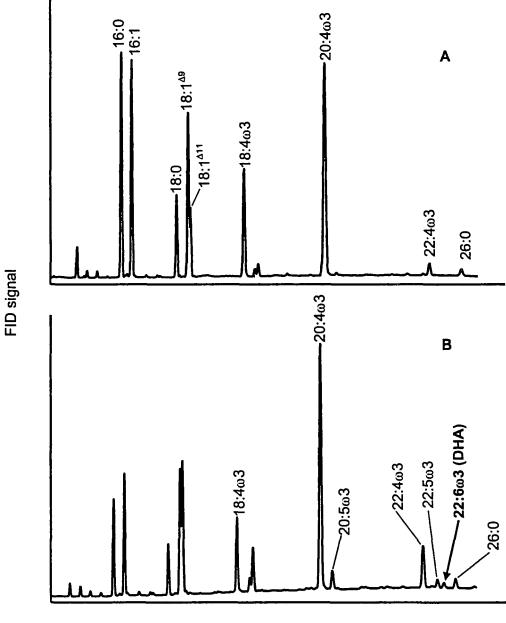


Figure 3: Reconstitution of DHA biosynthesis in yeast starting from 20:5ω3.



Retention time

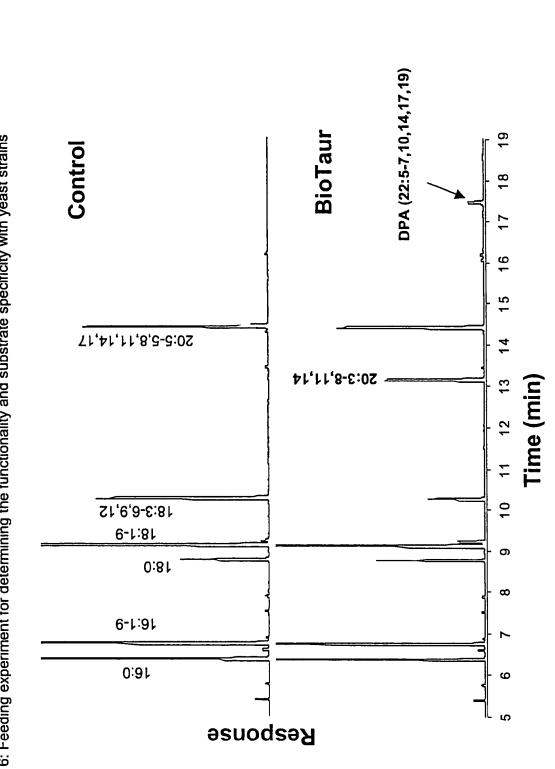
Figure 4: Reconstitution of DHA biosynthesis in yeast starting from 18:4ω3.



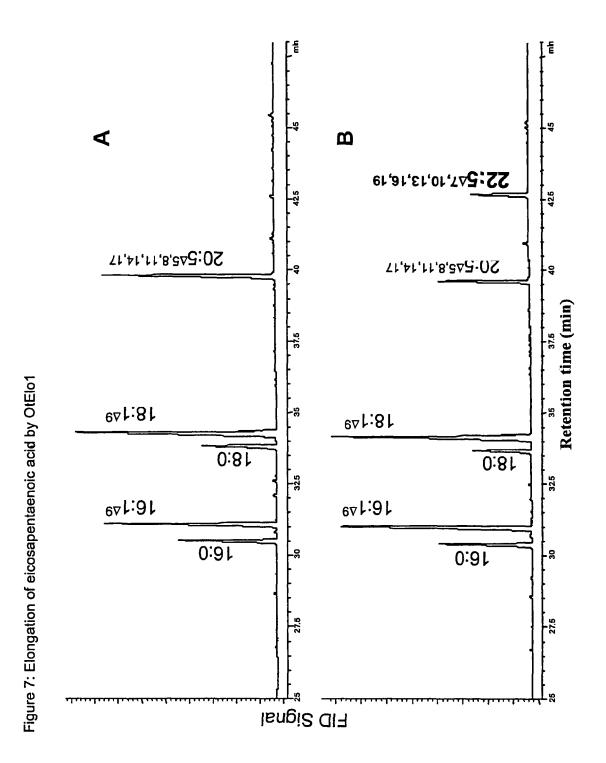
Retention time

Figure 5: Fatty acid composition (in mol%) of transgenic yeasts which had been transformed with the vectors pYes3-OmELO3/pYes2-EgD4 or pYes3-OmELO3/pYes2-EgD4+pESCLeu-PtD5. The yeast cells were cultured in minimal medium without tryptophan and uracil/ and leucin in the presence of 250 μM 20:5^{Δ5,8,11,14,17} and 18:4^{Δ6,9,12,15}, respectively. The fatty acid methyl esters were obtained from cell sediments by acid methanolysis and analyzed via GLC. Each value represents the mean (n=4) ± standard deviation.

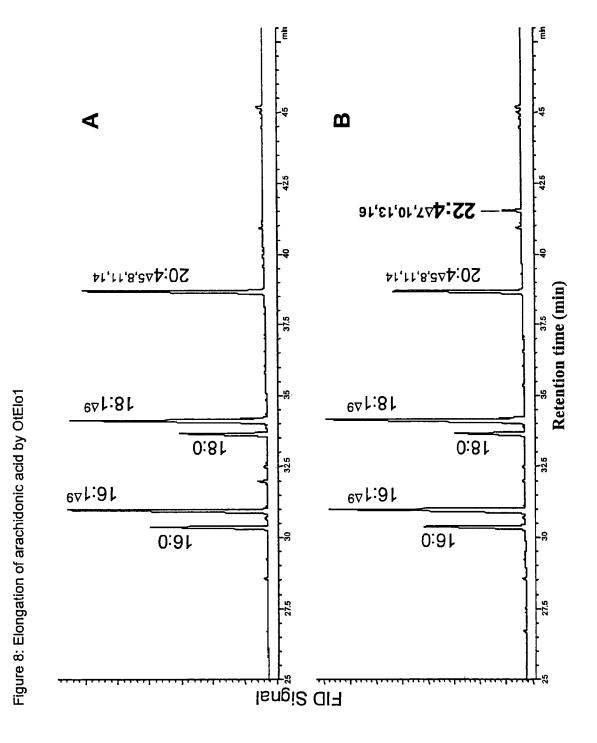
	pYes3-OmELO/pYes2-EgD4	pYes3-OmELO/pYes2-EgD4 EgD4 + pESCLeu-PtD5	
Fatty acids	Feeding of 20:5 ^{45,8,11,14,17}	Feeding of 18:4 ^{6,9,12,15}	
16:0	9.35 ± 1.61	7.35 ± 1.37	
⁴⁰ 16:1	14.70 ± 2.72	10.02 ± 1.81	
18:0	5.11 ± 1.09	4.27 ± 1.21	
18:1 ^{Δ9}	19.49 ± 3.01	10.81 ± 1.95	
18:1 ^{Δ11}	18.93 ± 2.71	11.61 ± 1.48	
18:4 ^{6,9,12,15}	-	7.79 ± 1.29	
20:1 ^{Δ11}	3.24 ± 0.41	1.56 ± 0.23	
20:1 ^{Δ13}	11.13± 2.07	$\textbf{4.40} \pm \textbf{0.78}$	
20:4^{68,11,14,17}	-	30.05 ± 3.16	
20:5 ^{\$5,8,11,14,17}	6.91± 1.10	3.72 ± 0.59	
22:4 ^{Δ10,13,16,17}	-	5.71 ± 1.30	
22:5 ^{47,10,13,16,19}	8.77 ± 1.32	1.10 ± 0.27	
22:6 ^{4,7,10,13,16,19}	2.73 ± 0.39	$\boldsymbol{0.58 \pm 0.10}$	

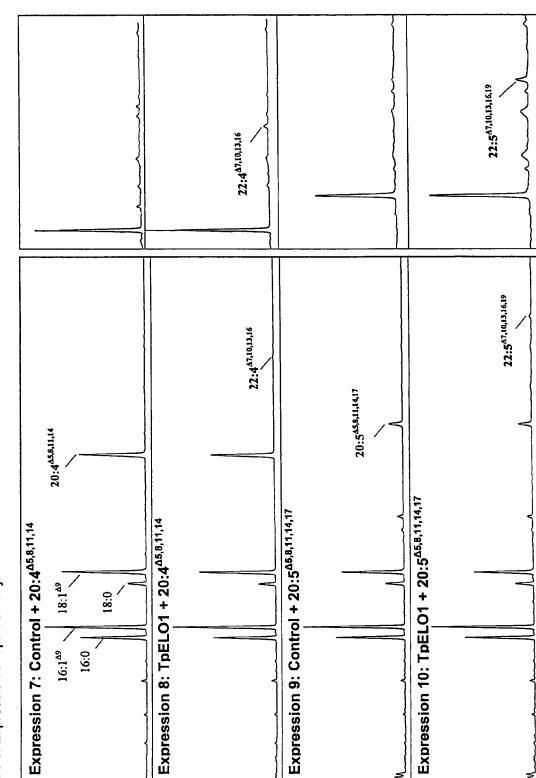






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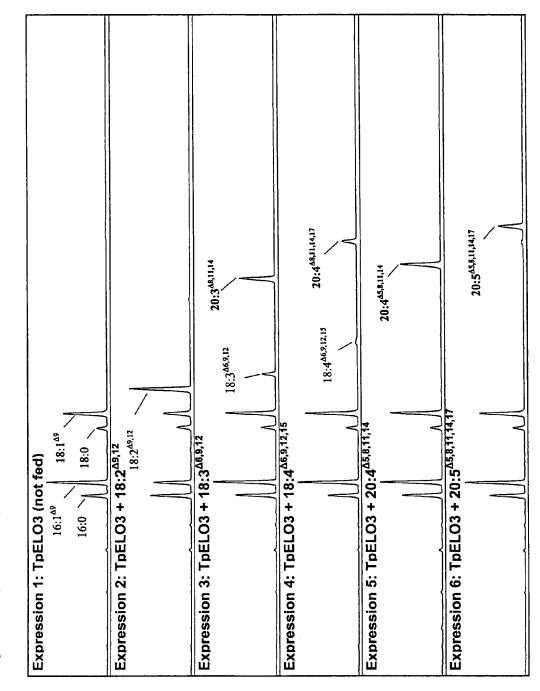
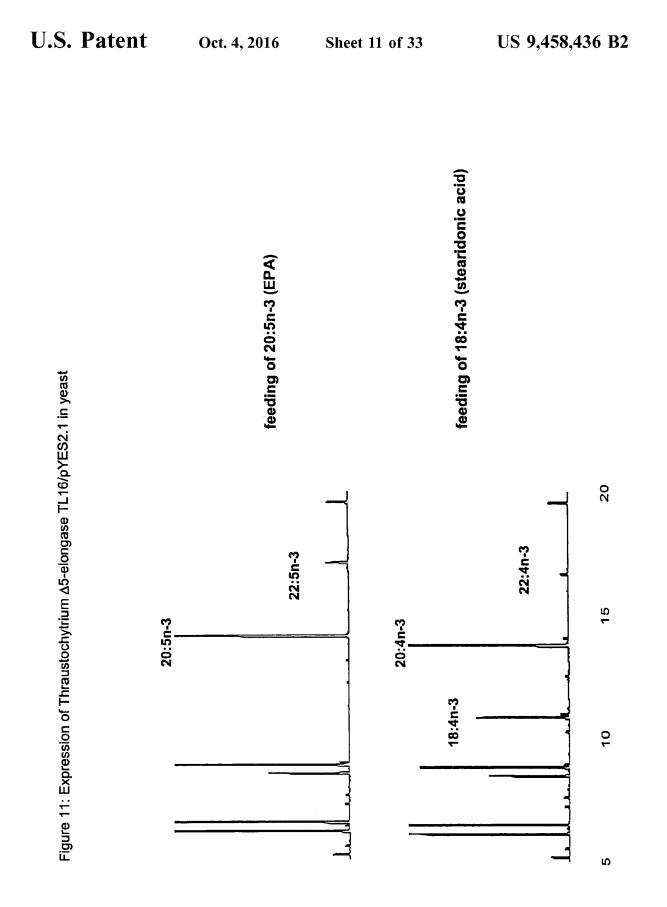


Figure 10: Expression of TpELO3 in yeast



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Figure 12: Desaturation of γ -linolenic acid (18:2 ω 6-fatty acid) to give α -linolenic acid (18:3 ω 3-fatty acid) by Pi-omega3Des.

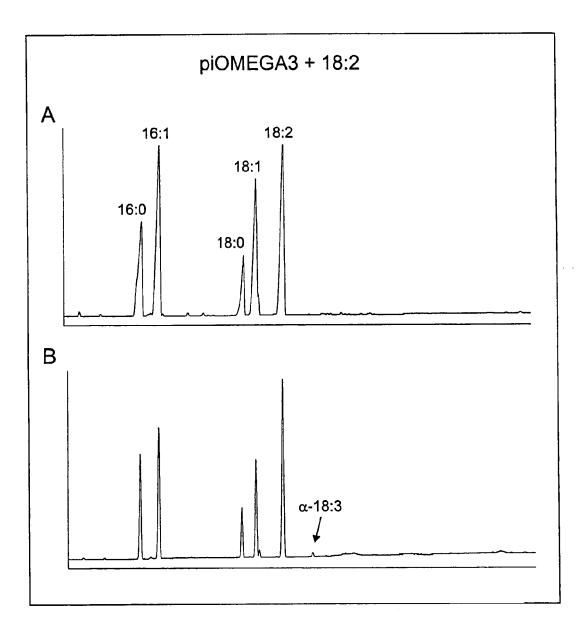


Figure 13: Desaturation of γ -linolenic acid (18:2 ω 6-fatty acid) to give stearidonic acid (18:4 ω 3-fatty acid) by Pi-omega3Des.

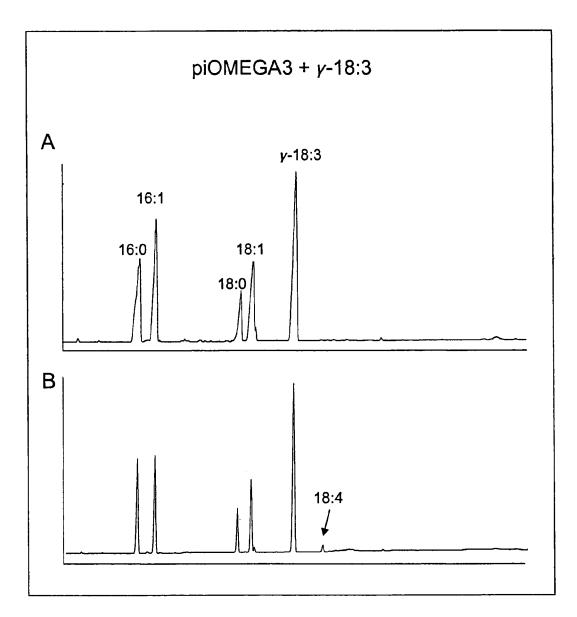


Figure 14: Desaturation of C20:2 ω6-fatty acid to give C20:3 ω3-fatty acid by Piomega3Des.

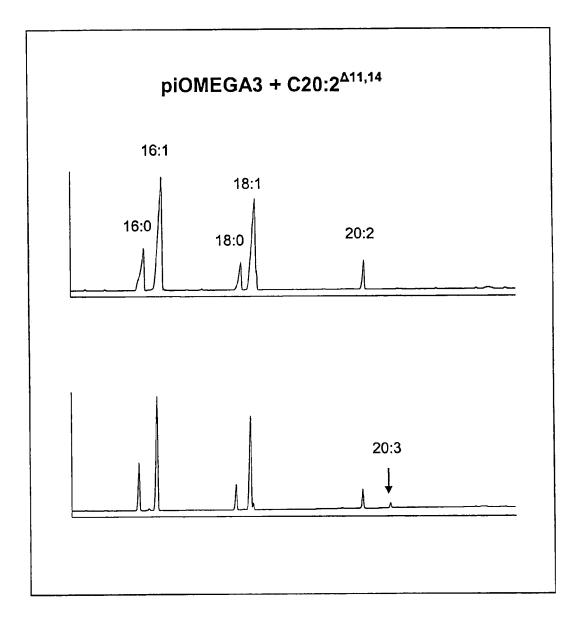


Figure 15: Desaturation of C20:3 ω 6-fatty acid to give C20:4 ω 3-fatty acid by Piomega3Des.

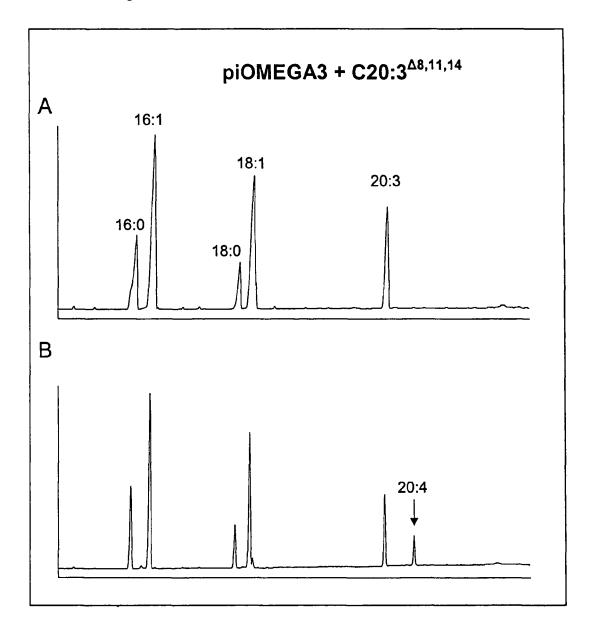


Figure 16: Desaturation of arachidonic acid (C20:4 ω6-fatty acid) to give eicosapentaenoic acid (C20:5 ω3-fatty acid) by Pi-omega3Des.

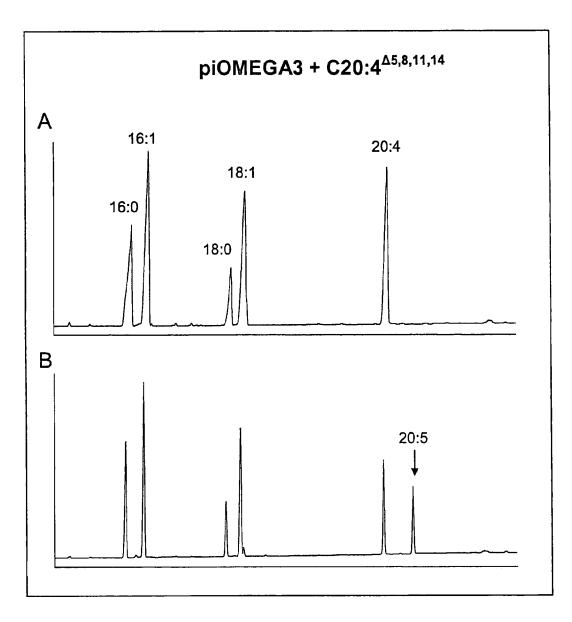
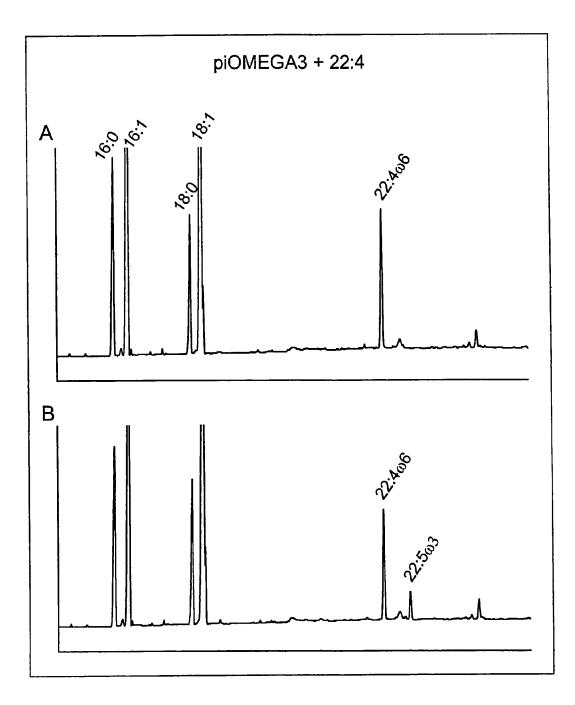


Figure 17: Desaturation of docosatetraenoic acid (C22:4 ω 6-fatty acid) to give docosapentaenoic acid (C22:5 ω 3-fatty acid) by Pi-omega3Des.



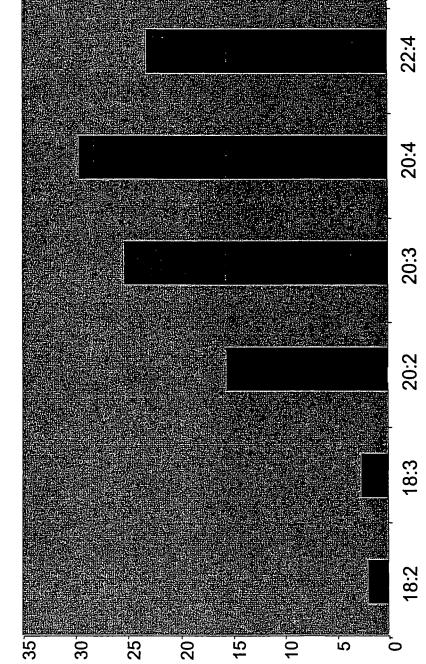


Figure 18: Substrate specificity of Pi-omega3Des with regard to different fatty acids

% Desaturation

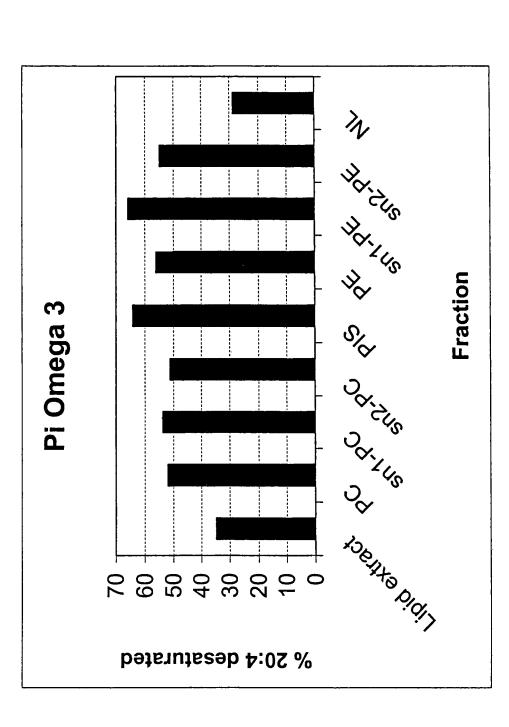
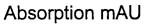
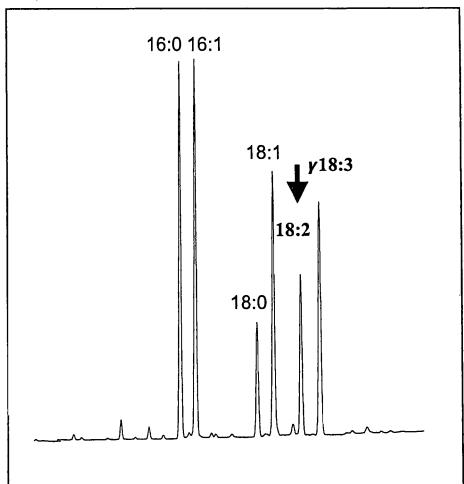


Figure 20: Conversion of linoleic acid (arrow) to give γ -linolenic acid (γ -18:3) by Ot-Des6.1.





Retention time

Figure 21: Conversion of linoleic acid and α -linolenic acid (A and C), and reconstitution of the ARA and EPA synthetic pathways, respectively, in yeast (B and D) in the presence of OtD6.1.

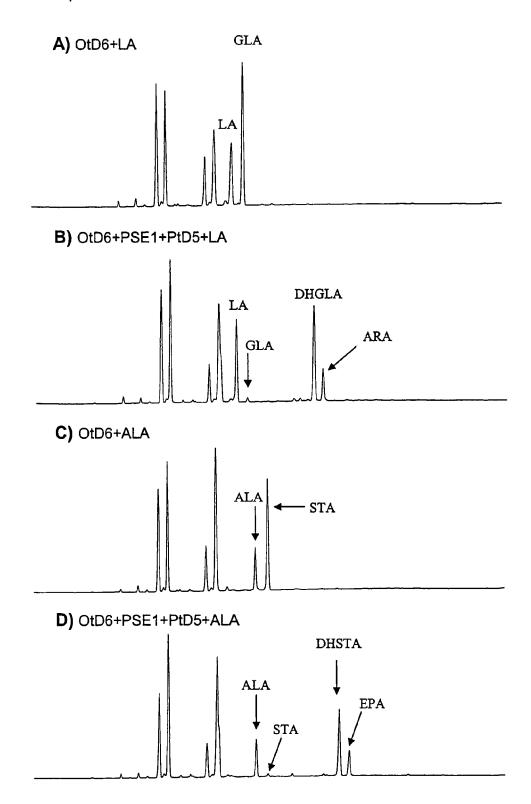


Figure 22: Expression of ELO(XI) in yeast

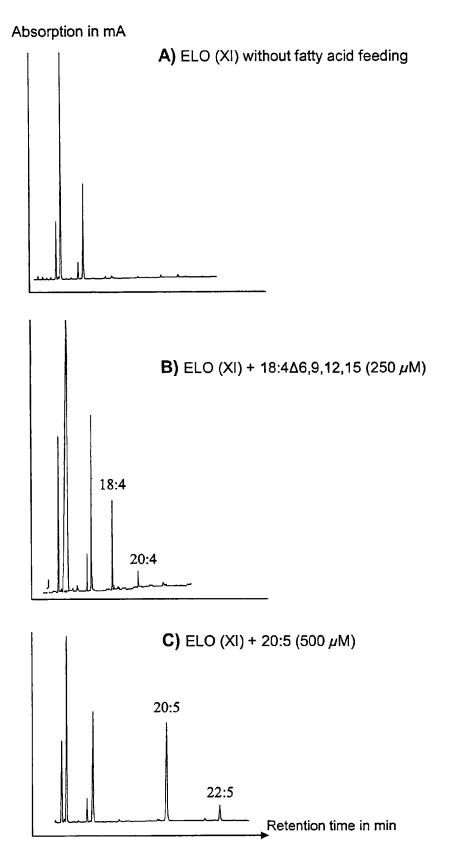


Figure 23:

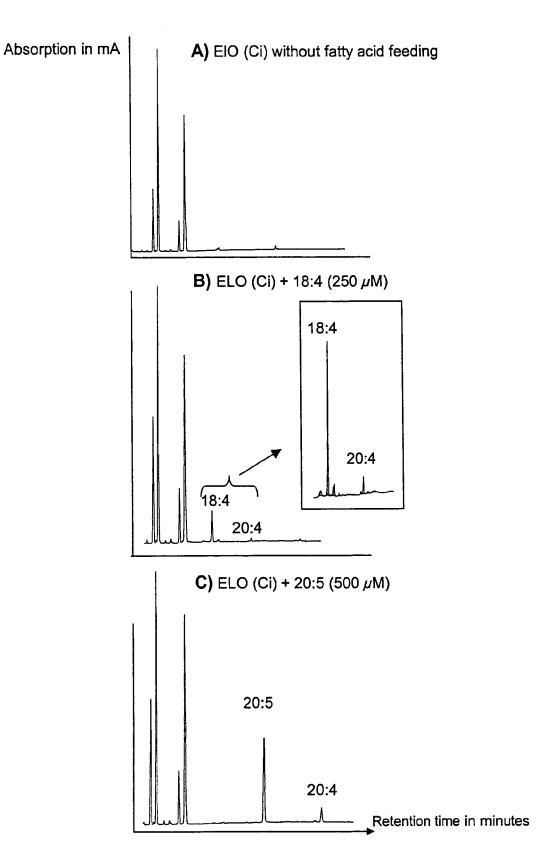


Figure 24: Elongation of eicosapentaenoic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22:5ω3).

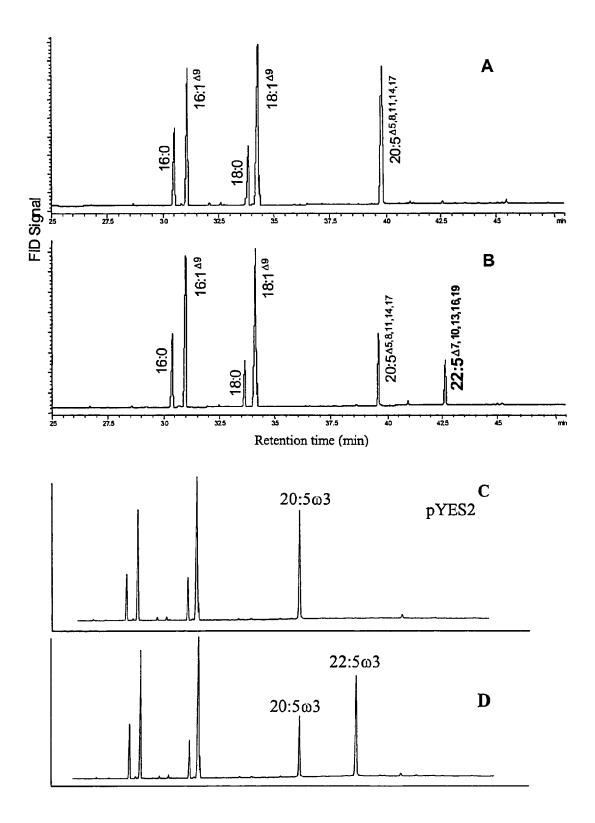


Figure 25:Elongation of arachidonic acid by OtElo1 (B) and OtElo1.2 (D), respectively.The controls (A, C) do not show the elongation product (22:4ω6).

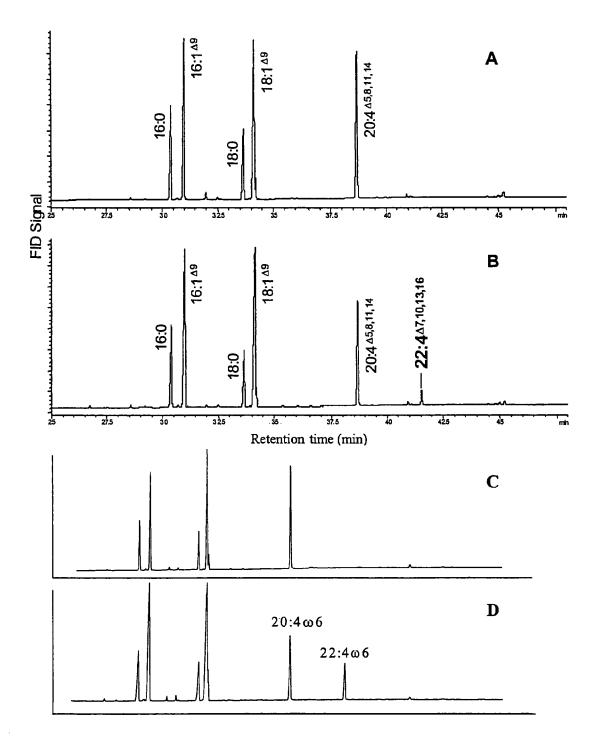
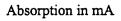
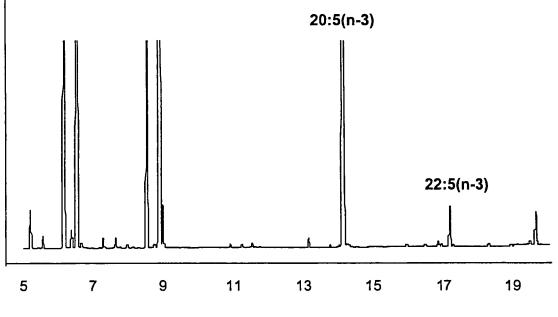


Figure 26: Elongation of 20:5n-3 by the elongases At3g06470.





Retention time in minutes

Figure 27: Substrate specificity of the Xenopus Elongase (A), Ciona Elongase (B) und Oncorhynchus Elongase (C)

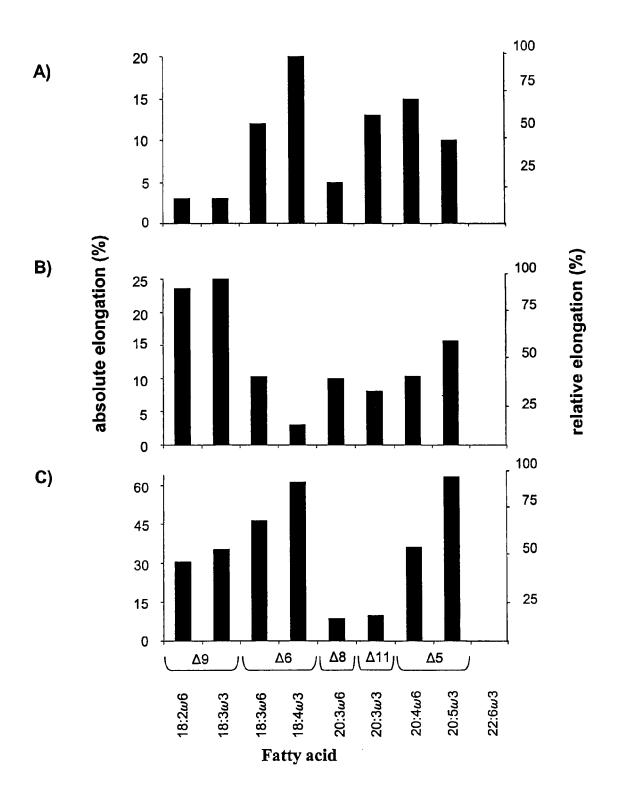


Figure 28: Substrate specificity of the Ostreococcus ∆5-elongase (A), the Ostreococcus ∆6-elongase (B), the Thalassiosira ∆5-elongase (C) and the Thalassiosira Ostreococcus ∆6-elongase (D)

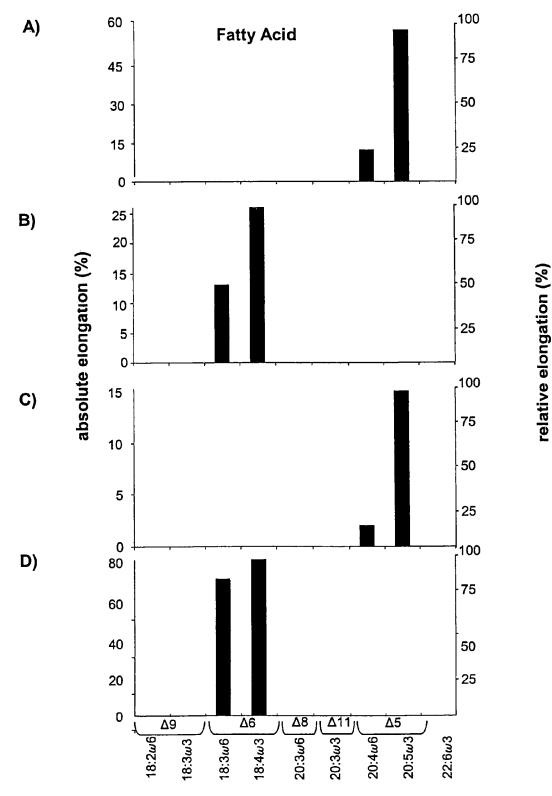


Figure 29: Expression of the Phaeodactylum tricornutum ∆6-elongase (PtELO6) in yeast. A) shows the elongation of the C18:3^{△6,9,12} fatty acid and B) the elongation of the C18:3^{△6,9,12,15} fatty acid

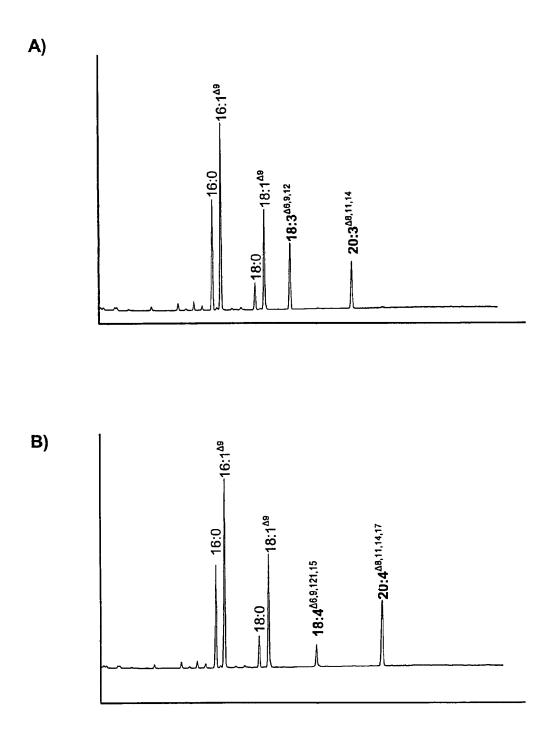
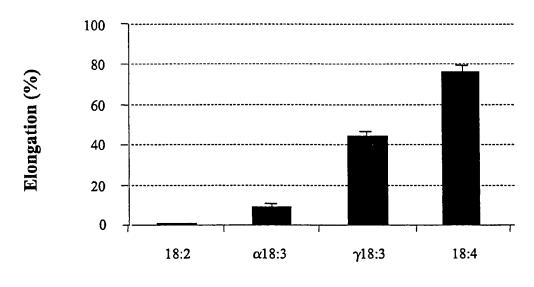
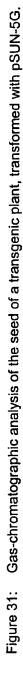


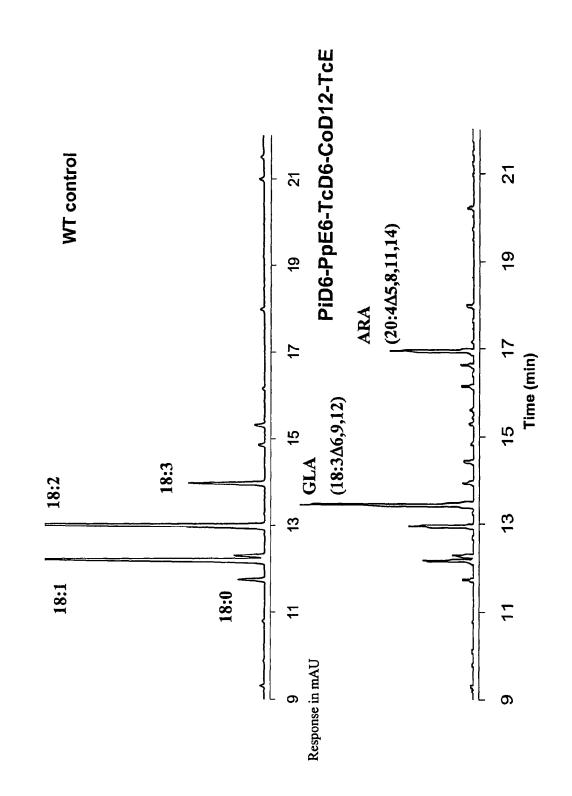
Figure 30: Figure 30 shows the substrate specificity of PtELO6 with regard to the substrates fed.



PtELO6 specificity

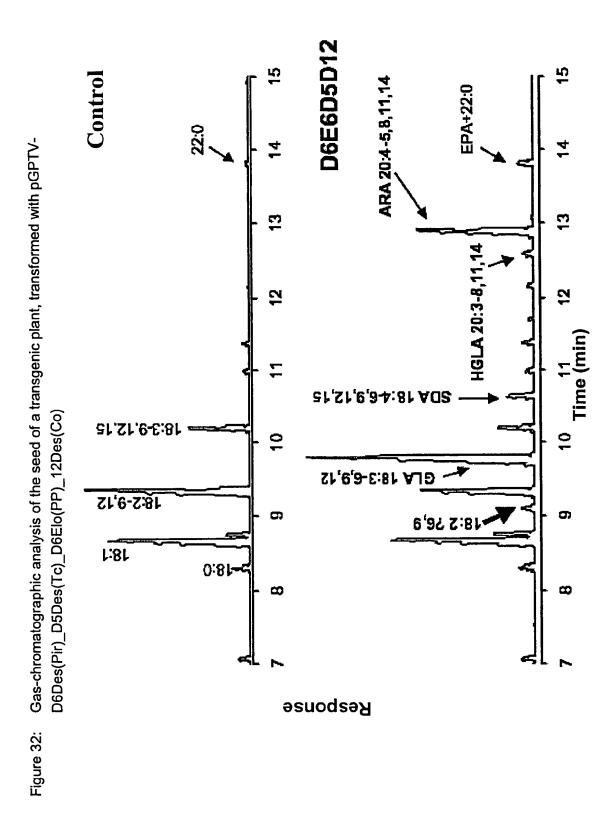
Fatty acid substrate

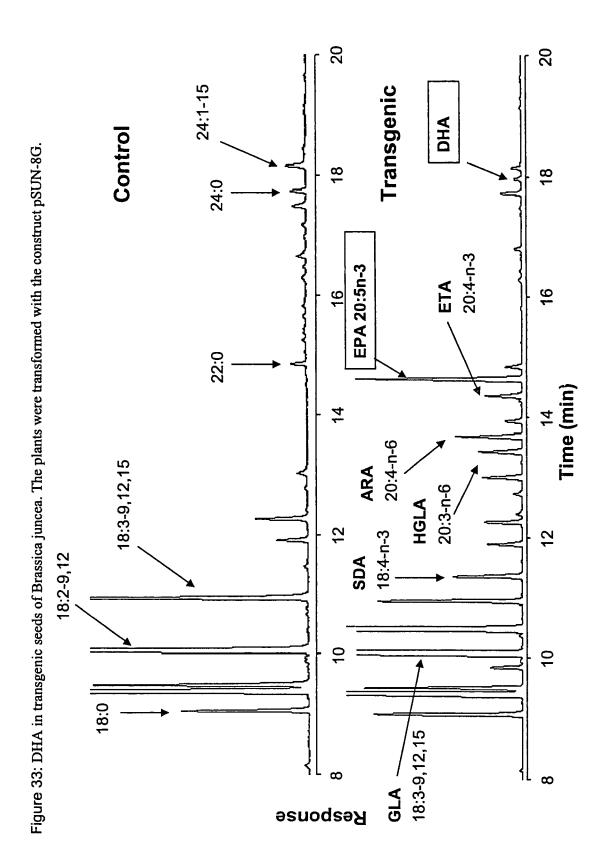




Oct. 4, 2016

U.S. Patent





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METHOD FOR PRODUCING POLYUNSATURATED FATTY ACIDS IN TRANSGENIC PLANTS

RELATED APPLICATIONS

This application is a national stage application (under 35 U.S.C. 371) of PCT/EP2005/001863 filed Feb. 23, 2005, and claims benefit of German application 10 2004 009 457.8 filed Feb. 27, 2004; German application 10 2004 012 370.5 ¹⁰ filed Mar. 13, 2004; German application 10 2004 017 518.7 filed Apr. 8, 2004; German application 10 2004 024 014.0 filed May 14, 2004; PCT application PCT/EP2004/07957 filed Jun. 16, 2004; and German application 10 2004 062 543.3 filed Dec. 24, 2004. ¹⁵

SUBMISSION ON COMPACT DISC

The contents of the following submission on compact discs are incorporated herein by reference in it s entirety: ²⁰ two copies of the Sequence Listing (COPY 1 and COPY 2) and a computer readable form copy of the Sequence Listing (CRF COPY), all on compact disc, each containing: file name: "Sequence Listing-13987-00020-US", date recorded: May 9, 2007, size: 613 KB. 25

FIELD OF THE INVENTION

The present invention relates to a process for the production of polyunsaturated fatty acids in the seed of transgenic 30 plants by introducing, into the organism, nucleic acids which encode polypeptides with ω 3-desaturase, Δ 12-desaturase, Δ 6-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase and/or Δ 4-desaturase activity, preferably polypeptides with Δ 6-desaturase, Δ 6-elongase and Δ 5-desaturase activity. 35

The nucleic acid sequences are the sequences shown in SEQ ID NO: 11, SEQ ID NO: 27, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 and SEQ ID NO: 201. Preferably, a further nucleic acid sequence which encodes a polypeptide with a Δ 12-desaturase activity is additionally 40 introduced into the plant, in addition to these nucleic acid sequences, and also expressed simultaneously. Especially preferably, this is the nucleic acid sequence shown in SEQ ID NO: 195.

These nucleic acid sequences can advantageously be 45 expressed in the organism, if appropriate together with further nucleic acid sequences which encode polypeptides of the biosynthesis of the fatty acid or lipid metabolism. Especially advantageous are nucleic acid sequences which encode a $\Delta 6$ -desaturase, a $\Delta 5$ -desaturase, $\Delta 4$ -desaturase, 50 $\Delta 12$ -desaturase and/or $\Delta 6$ -elongase activity. These desaturases and elongases originate advantageously from *Thalassiosira, Euglena* or *Ostreococcus*. Furthermore, the invention relates to a process for the production of oils and/or triacylglycerides with an elevated content of long-chain 55 polyunsaturated fatty acids.

In a preferred embodiment, the invention furthermore relates to a process for the production of arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid and to a process for the production of triglycerides with an elevated 60 content of unsaturated fatty acids, in particular arichidonic acid, eicosapentaenoic acid and/or docosahexaenoic acid, in transgenic plants, advantageously in the seed of the transgenic plant. The invention relates to the generation of a transgenic plant with an elevated content of polyunsaturated 65 fatty acids, in particular arichidonic 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.

The invention furthermore relates to recombinant nucleic acid molecules comprising the nucleic acid sequences which encode the polypeptides with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase and $\Delta 5$ -elongase activity, either jointly or individually, and transgenic plants which comprise the abovementioned recombinant nucleic acid molecules.

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. Moreover, the invention relates to unsaturated fatty acids and to triglycerides with an elevated content of unsaturated fatty acids and to their use.

DESCRIPTION OF RELATED ART

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3phosphate, 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 25 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 35 Press: Washington, D.C., p. 612-636 and references cited therein; Lengeler et al. (Ed.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, and the references therein, and Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 and the references therein). To undergo the further elongation steps, the resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This is made possibly by acyl-CoA:lysophospholipid acyltransferases. Moreover, these enzymes are capable of transferring the elongated fatty acids from the CoA esters back to the phospholipids. If appropriate, this reaction sequence can be followed repeatedly.

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

With regard to publications on 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 and cofactors and the storage and assembly of triacylglycerol, including the references cited therein, see the following papers: Kinney, 1997, Genetic Engineering, Ed.: J K 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.: J K 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, Eds.: Murata and Somerville, Rockville, American Society of Plant Physiologists, 150-158, Murphy & Ross 1998, Plant Journal. 13(1): 1-16.

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).

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and the pharmacological sector. Depending on 10 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. Polyunsaturated fatty acids such as linoleic and linolenic acid are essential for mammals since they cannot 15 be produced by the latter. This is why polyunsaturated ω3-fatty acids and ω6-fatty acids are an important constituent of human and animal food. Thus, for example, lipids with unsaturated fatty acids, specifically with polyunsaturated fatty acids, are preferred in human nutrition. The 20 polyunsaturated w3-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 w3-fatty acids to the food (Shimikawa 2001, World 25 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 30 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.

 ω 3- and ω 6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomo-y-linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosa- 40 pentaenoic acid. Eicosanoids (known as the PG_2 series) which are formed from the w6-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 eicosa-pentaenoic 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, 50 the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A Lipids 30:1-14, 1995; Horrocks, L A and Yeo Y K Pharmacol Res 40:211-225, 1999). There is therefore a demand for the production 55 of polyunsaturated long-chain fatty acids.

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 60 such as docosahexaenoic acid (=DHA, C22:6^{Δ 4,7,10,13,16,19}) or eicosapentaenoic acid (=EPA, C20:5 $^{\Delta5,8,11,14,17}$) are added to infant formula to improve the nutritional value. The unsaturated fatty acid DHA is supposed to have a positive effect on the development and maintenance of brain func-65 tion. There is therefore a demand for the production of polyunsaturated long-chain fatty acids.

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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, algae such as Crypthecodinium or Phaeodactylum and others, being obtained, as a rule, in the form, of their triacylglycerides (=triglycerides=triglycerols). However, they can also be obtained from animals, for example, fish. The free fatty acids are advantageously prepared by hydrolysis. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (ARA, C20:4^{Δ 5,8,11,14}), dihomo- γ -linolenic acid (C20:3^{Δ 8,11,14}) or docosapentaenoic acid (DPA, C22:5^{47,10,13,16,19}) 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.

Depending on the intended use, oils with saturated or unsaturated fatty acids are preferred. In human nutrition, for example, lipids with unsaturated fatty acids, specifically polyunsaturated fatty acids, are preferred. The polyunsaturated ω 3-fatty acids are said to have a positive effect on the cholesterol level in the blood and thus on the possibility of preventing heart disease. The risk of heart disease, stroke or hypertension can be reduced markedly by adding these ω 3-fatty acids to the food. Also, ω 3-fatty acids have a positive effect on inflammatory, specifically on chronically inflammatory, processes in association with immunological diseases such as rheumatoid arthritis. They are therefore added to foodstuffs, specifically to dietetic foodstuffs, or are employed in medicaments. w3-fatty acids such as arachidonic acid tend to have an adverse effect on these disorders in connection with these rheumatic diseases on account of 35 our usual dietary intake.

Owing to their positive characteristics, 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 desaturates are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144-20149, Wada et al., Nature 347, 1990: 200-203 or Huang et al., Lipids 34, 1999: 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., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 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. $\Delta 6$ -Desaturases are described in WO 93/06712, U.S. Pat. No. 5,614,393, U.S. Pat. No. 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 is 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

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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) 5 describe the characterization of plant elongases for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the formation of waxes in plants (C_{28} - C_{32}). The synthesis of arachidonic acid and EPA is described, for example, in WO 10 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 WO 02/44320.

Especially suitable microorganisms for the production of 15 PUFAs are microorganisms such as microalgae such as Phaeodactylum tricornutum, Porphiridium species, Thraustochytrium species, Schizochytrium species or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungi such as Mortierella, Entomophthora or Mucor and/or 20 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 devel- 25 opment 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 adds is a time-consuming 30 and difficult process, which is why as described above, recombinant methods are preferred. However, 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; where, as a rule, they are 35 generally obtained as fatty acid mixtures, depending on the microorganisms used.

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 40 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 oilseed crops such 45 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 oilseed crops, genes which 50 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, $\Delta 5$ -elongases or $\Delta 4$ -desaturases. These genes can advantageously be isolated from microorganisms and lower 55 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 *Phy*scomitrella patens and $\Delta 6$ -elongase genes from P. patens and from the nematode C. elegans. A variety of synthetic 60 pathways is being discussed for the synthesis of arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (FIG. 1). Thus, EPA or DHA are produced in marine bacteria such as Vibrio sp. or Shewanella sp. via the polyketide pathway (Yu, R. et al. Lipids 35:1061-1064, 65 2000; Takeyama, H. et al. Microbiology 143:2725-2731, 1997).

An alternative strategy is the alternating activity of desaturases and elongases (Zank, T. K. et al. Plant Journal 31:255-268, 2002; Sakuradani, E. et al. Gene 238:445-453, 1999). A modification of the above-described pathway by $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and $\Delta 4$ -desaturase is the Sprecher pathway (Sprecher 2000, Biochim. Biophys. Acta 1486:219-231) in mammals. Instead of the $\Delta 4$ -desaturation, a further elongation step is effected here to give C24, followed by a further $\Delta 6$ -desaturation and finally β -oxidation to give the C₂₂ chain length. Thus what is known as Sprecher pathway (see FIG. 1) is, however, not suitable for the production in plants and microorganisms since the regulatory mechanisms are not known.

Depending on-their desaturation pattern, the polyunsaturated fatty acids can be divided into two large classes, viz ω 6- or ω 3-fatty acids, which differ with regard to their metabolic and functional activities (FIG. 1).

The starting material for the ω 6-metabolic pathway is the fatty acid linoleic acid $(18:2^{\Delta 9,12})$ while the ω 3-pathway proceeds via linolenic acid $(18:3^{\Delta 9,12,15})$. Linolenic acid is formed 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).

Mammals, and thus also humans, have no corresponding desaturase activity ($\Delta 12$ - and $\omega 3$ -desaturase) and must 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 $\omega 3$ -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 the sequence of desaturase and elongase reactions. The application of $\omega 3$ -fatty acids shows the therapeutic activity described above in the treatment of cardiovascular diseases (Shimikawa 2001, World Rev. Nutr. Diet. 88, 100-108), inflammations (Calder 2002, Proc. Nutr. Soc. 61, 345-358) and arthritis (Cleland and James 2000, J. Rheumatol. 27, 2305-2307).

From the angle of nutritional physiology, it is therefore advantageous to achieve a shift between the ω 6-synthetic pathway and the ω -3-synthetic pathway (see FIG. 1) so that more ω 3-fatty acids are produced. The enzymatic activities of various ω 3-desaturases which desaturate C_{18:2}-, C_{22:4}- or C_{22:5}-fatty acids have been described in the literature (see FIG. 1). However, none of the desaturases whose biochemistry has been described converts a broad range of substrates of the ω 6-synthetic pathway into the corresponding fatty acids of the ω 3-synthetic pathway.

The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C_{20} - and C_{22} -PUFAs, respectively. This process proceeds via 4 steps. The first step is the condensation of malonyl-CoA onto the fatty-acid-acyl-CoA by ketoacyl-CoA synthase (KCS, here-inbelow 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).

No specific elongase has been described to date for the production of DHA (C22:6 n-3) in organisms which do not naturally produce this fatty acid. Only elongases which provide C_{20^-} or C_{24} -fatty acids have been described to date. A Δ 5-elongase activity has not been described to date.

The first transgenic plants which comprise and express genes encoding LCPUFA biosynthesis enzymes and which, as a consequence, produce LCPUFAs were described for the first time, for example, in DE-A-102 19 203 (Process for the production of polyunsaturated fatty acids in plants) or in WO 2004/071467. However, these plants produce LCPUFAs in amounts which require further optimization for processing the oils which are present in the plants. Thus, ARA content in the plants described in DE-A-102 19 203 only amounts to 0.4 to 2% and the EPA content only to 0.5 to 1%, in each case based on the total lipid content of the plants. WO 2004/ 071467 discloses higher contents of polyunsaturated C₂₀and C222-fatty acids such as ARA, EPA or DHA. However, the process disclosed has a series of grave disadvantages. It seems that DHA cannot be detected at all in the seeds in the process disclosed. To produce PUFAs, soybean is less suitable, owing to its low oil content of approximately only 20% by weight. Soybean is an advantageous protein source and is therefore grown on a large scale. However, the oil content of soybeans is rather low. Moreover, the dihomo-y-linolenic acid (=DGHL or HGLA) content obtained in the production 20 process is much too high. HGLA is hardly detectable in fish oils or algal oils or microbial oils. A further disadvantage is that the plants disclosed in WO 2004/071467 were generated by cotransformation, which leads to the segregation of the characteristics in the subsequent generations, and thus to an 25 increased selection effort.

To make possible the fortification of food and/or of feed with these polyunsaturated fatty acids, there is therefore a great need for a simple, inexpensive process for the production of these polyunsaturated fatty acids in plant systems, ³⁰ especially in the seed of transgenic plants.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows various synthetic pathways for the biosyn- 35 thesis of DHA (docosahexaenoic acid).

FIG. **2** shows substrate specificity of the 5-elongase (SEQ ID NO: 53) with regard to different fatty acids.

FIG. **3** shows reconstitution of DHA biosynthesis in yeast starting from $20:5\omega 3$.

FIG. 4 shows reconstitution of DHA biosynthesis in yeast starting from $18:4\omega 3$.

FIG. **5** shows fatty acid composition (in mol %) of transgenic yeasts which had been transformed with the vectors pYes3-OmELO3/pYes2-EgD4 or pYes3-OmELO3/ 45 pYes2-EgD4+pESCLeu-PtD5. The yeast cells were cultured in minimal medium without tryptophan and uracil/and leucin in the presence of 250M $20:5^{\Delta5,8,11,14,17}$ and $18:4^{\Delta6,9,12}$, 15, respectively. The fatty acid methyl esters were obtained from cell sediments by acid methanolysis and analyzed via 50 GLC. Each value represents the mean (n=4)±standard deviation.

FIG. 6 shows feeding experiment for determining the functionality and substrate specificity with yeast strains.

FIG. 7 shows elongation of eicosapentaenoic acid by 55 OtElo1.

FIG. 8 shows elongation of arachidonic acid by OtElo1.

FIG. 9 shows expression of TpELO1 in yeast.

FIG. 10 shows expression of TpELO3 in yeast.

FIG. **11** shows expression of *Thraustochytrium* 5-elon- 60 gase TL16/pYES2.1 in yeast.

FIG. **12** shows desaturation of γ -linolenic acid (18:2 ω 6-fatty acid) to give α -linolenic acid (18:3 ω 3-fatty acid) by Pi-omega3Des.

FIG. 13 shows desaturation of γ -linolenic acid (18:2 65 ω 6-fatty acid) to give stearidonic acid (18:4 ω 3-fatty acid) by Pi-omega3Des.

FIG. 14 shows desaturation of C20:2 ω 6-fatty acid to give C20:3 ω 3-fatty acid by Pi-omega3Des.

FIG. 15 shows desaturation of C20:3 ω 6-fatty acid to give C20:4 ω 3-fatty acid by Pi-omega3Des.

FIG. **16** shows desaturation of arachidonic acid (C20:4 ω 6-fatty acid) to give eicosapentaenoic acid (C20:5 ω 3-fatty acid) by Pi-omega3Des.

FIG. 17 shows desaturation of docosatetraenoic acid (C22:4 ω 6-fatty acid) to give docosapentaenoic acid (C22:5 ω 3-fatty acid) by Pi-omega3Des.

FIG. 18 shows substrate specificity of Pi-omega3Des with regard to different fatty acids.

FIG. **19** shows desaturation of phospholipid-bound arachidonic acid to give EPA by Pi-Omega3Des.

FIG. **20** shows conversion of linoleic acid (arrow) to give γ -linolenic acid (γ -18:3) by OtDes6.1.

FIG. **21** shows conversion of linoleic acid and α -linolenic acid (A and C), and reconstitution of the ARA and EPA synthetic pathways, respectively, in yeast (B and D) in the presence of OtD6.1.

FIG. 22 shows expression of ELO(XI) in yeast.

FIG. 23 shows substrate specificity of ELO(Ci).

FIG. **24** shows elongation of eicosapentaenoic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product $(22:5\omega 3)$.

FIG. **25** shows elongation of arachidonic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product $(22:4\omega 6)$.

FIG. **26** shows elongation of 20:5n-3 by the elongases At3g06470.

FIG. **27** shows substrate specificity of the *Xenopus* Elongase (A), Ciona Elongase (B) and *Oncorhynchus* Elongase (C).

FIG. **28** shows substrate specificity of the *Ostreococcus* Δ 5-elongase (A), the *Ostreococcus* Δ 6-elongase (B), the *Thalassiosira* Δ 5-elongase (C) and the *Thalassiosira* Δ 6-elongase (D).

FIG. **29** shows expression of the *Phaeodactylum tricor*nutum Δ 6-elongase (PtELO6) in yeast. A) shows the elongation of the C18:3^{Δ 6,9,12} fatty acid and B) the elongation of the C18:3^{Δ 6,9,12,15} fatty acid.

FIG. **30** shows the substrate specificity of PtELO6 with regard to the substrates fed.

FIG. **31** shows gas-chromatographic analysis of the seed of a transgenic plant, transformed with pSUN-5G.

FIG. **32** shows gas-chromatographic analysis of the seed of a transgenic plant, transformed with pGPTV-D6Des (Pir)_D5Des(Tc)_D6Elo(PP)_12Des(Co).

FIG. **33** shows DHA in transgenic seeds of *Brassica juncea*. The plants were transformed with the construct pSUN-8G.

DETAILED DESCRIPTION OF THE INVENTION

The object of the invention was therefore to develop a process for the production of large amounts of polyunsaturated fatty acids, specifically ARA, EPA and DHA, in the seed of a transgenic plant. This object was achieved by the process according to the invention for the production of compounds of the general formula I

 \mathbb{R}^{1} $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$ $\mathbb{C}^{H_{2}}$

(I)

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in the seeds of transgenic plants with a content of at least 20% by weight based on the total lipid content, which comprises the following process steps:

- a) introducing, into the organism, at least one nucleic acid sequence which encodes a $\Delta 9$ -elongase and $\Delta 6$ -desaturase ⁵ activity, and
- b) introducing, into the organism, at least one nucleic acid sequence which encodes a $\Delta 8$ -desaturase and $\Delta 6$ -elongase activity, and
- c) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ 5-desaturase activity, and
- d) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ5-elongase activity, and
- e) introducing, into the organism, at least one nucleic acid $_{15}$ sequence which encodes a $\Delta4\text{-desaturase}$ activity, and

where the variables and substituents in formula I have the following meanings:

R¹=hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II

$$\begin{array}{c} H_2C \longrightarrow O \longrightarrow R^2 \\ H_C \longrightarrow O \longrightarrow R^3 \\ H_2C \longrightarrow O \longrightarrow \end{array}$$
(II)

- R²=hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphati-35 dylinositol or saturated or unsaturated C₂-C₂₄alkylcarbonyl,
- R^3 =hydrogen, saturated or unsaturated C_2 - C_{24} -alkylcarbonyl, or R^2 and R^3 independently of one another are a radical of the formula Ia: 40

$$(Ia)$$

n=2, 3, 4, 5, 6, 7 or 9, m=2, 3, 4, 5 or 6 and p=0 or 3.50 Advantageously, the variables n, m and p in the abovementioned formula I and Ia denote the following: n=2, 3 or 5, m=4, 5 or 6 and p=0 or 3. In an especially advantageous embodiment of the process; the variables n, m and p in the formulae I and Ia denote the following: m=4, n=3, p=3 and the compounds of the general formula I and Ia thus denote arachidonic acid, and/or m=5, n=3, p=0 and the compounds of the general formula I and Ia thus denote eicosapentaenoic acid, and/or m=5, n=5, p=0 and the compounds of the general formula I and Ia thus denote docosapentaenoic acid 60 is and/or m=6, n=3, p=0 and the compounds of the general formula I and Ia thus denote docosahexaenoic acid is. R^1 in the general formula I is hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidyl-65 glycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II

$$H_2C \longrightarrow O \longrightarrow R^2$$

$$H_2C \longrightarrow O \longrightarrow R^3$$

$$H_2C \longrightarrow O \longrightarrow C$$

The abovementioned radicals of R^1 are always bonded to the compounds of the general formula I in the form of their thioesters.

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 $\rm R^2$ in the general formula II is hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated $\rm C_2-C_{24}$ -alkylcarbonyl.

Alkyl radicals which may be mentioned are substituted or unsubstituted, saturated or unsaturated C2-C24-alkylcarbonyl chains such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl, n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octa-25 decylcarbonyl-, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl- or n-tetracosanylcarbonyl, which comprise one or more double bonds. Saturated or unsaturated C10-C22-alkylcarbonyl radicals such as n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcar-30 bonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbdnyl or h-tetracosanylcarbbnyl, which comprise one or more double bonds are preferred. Especially preferred are saturated and/or unsaturated C_{10} - C_{22} -alkylcarbonyl radicals such as C_{10} -alkylcarbonyl, C_{11} -alkylcarbonyl, C_{12} -alkylcarbonyl, C_{13} -alkylcarbonyl, C_{14} -alkylcarbonyl, C_{16} alkylcarbonyl, C18-alkylcarbonyl, C20-alkylcarbonyl or C22alkylcarbonyl radicals which comprise one or more double bonds. Very especially preferred are saturated or unsaturated C₁₆-C₂₂-alkylcarbonyl radicals such as C₁₆-alkylcarbonyl, $\mathrm{C}_{18}\text{-alkylcarbonyl},\,\mathrm{C}_{20}\text{-alkylcarbonyl}$ or $\mathrm{C}_{22}\text{-alkylcarbonyl}$ radicals which comprise one or more double bonds. These advantageous radicals can comprise two, three, four, five or 45 six double bonds. The especially preferred radicals with 20 or 22 carbon atoms in the fatty acid chain comprise up to six double bonds, advantageously three, four, five or six double bonds, especially preferably four, five or six double bonds, very especially preferably five or six. All the abovementioned radicals are derived from the corresponding fatty acids.

 R^3 in the formula II is hydrogen, saturated or unsaturated C_2 - C_{24} -alkylcarbonyl.

Alkyl radicals which may be mentioned are substituted or 55 unsubstituted, saturated or unsaturated C2-C24-alkylcarbonyl chains such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl-, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl, n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl-, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl- or n-tetracosanylcarbonyl, which comprise one or more double bonds. Saturated or unsaturated C10-C22-alkylcarbonyl radicals such as n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl,

(II)

n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, which comprise one or more double bonds are preferred. Especially preferred are saturated and/or unsaturated C₁₀-C₂₂-alkylcarbonyl radi- 5 cals such as $\mathrm{C}_{10}\text{-alkylcarbonyl},~\mathrm{C}_{11}\text{-alkylcarbonyl},~\mathrm{C}_{12}\text{-}$ alkylcarbonyl, C_{13} -alkylcarbonyl, C_{14} -alkylcarbonyl, C_{16} alkylcarbonyl, $\rm C_{18}$ -alkylcarbonyl, $\rm C_{20}$ -alkylcarbonyl or $\rm C_{2}$ alkylcarbonyl radicals which comprise one or more double bonds. Very especially preferred are saturated or unsaturated 10 C16-C22-alkylcarbonyl radicals such as C16-alkylcarbonyl, C18-alkylcarbonyl, C20-alkylcarbonyl or C22-alkylcarbonyl radicals which comprise one or more double bonds. These advantageous radicals can comprise two, three, four, five or six double bonds. The especially preferred radicals with 20 15 or 22 carbon atoms in the fatty acid chain comprise up to six double bonds, advantageously three, four, five or six double bonds, especially preferably four, five or six double, bonds, very especially preferably five or six. All the abovementioned radicals are derived from the corresponding fatty 20 acids.

The abovementioned radicals of R^1 , R^2 and R^3 can be substituted by hydroxyl and/or epoxy groups and/or can comprise triple bonds.

The polyunsaturated fatty acids produced in the process 25 according to the invention advantageously comprise at least two, advantageously three, four, five or six, double bonds. The fatty acids especially advantageously comprise four, five or six double bonds. Fatty acids produced in the process advantageously have 18, 20 or 22 C atoms in the fatty acid chain; the fatty acids preferably comprise 20 or 22 carbon atoms in the fatty acid chain. Saturated fatty acids are advantageously reacted to a minor degree, or not at all, by the nucleic acids used in the process. To a minor degree is to be understood as meaning that the saturated fatty acids are 35 reacted with less than 5% of the activity, advantageously less than 3%, especially advantageously with less than 2%, very especially preferably with less than 1, 0.5, 0.25 or 0.125% of the activity in comparison with polyunsaturated fatty acids. These fatty acids which have been produced can be 40 produced in the process as a single product or be present in a fatty acid mixture.

The nucleic acid sequences used in the process according to the invention take the form of isolated nucleic acid sequences which encode polypeptides with $\Delta 9$ -elongase, 45 $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 4$ -desaturase activity.

Nucleic acid sequences which are advantageously used in the process according to the invention are nucleic acid sequences which encode polypeptides with $\Delta 9$ -elongase, 50 $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 4$ -desaturase activity selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 55 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ 60 ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49; SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID 65 NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91,

SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201, or

- b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEO ID NO: 70, SEO ID NO: 72, SEO ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 184, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200 or SEQ ID NO: 202, or
- 30 c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO:113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193; SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201, which encode polypeptides with at least 40% identity at the amino acid level with SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO; 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120,

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SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 184, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200 or SEQ ID NO: 202 and which have a Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase or Δ 4-de- ⁵ saturase activity.

Advantageously, the substituents R^2 or R^3 in the general formulae I and II independently of one another are saturated or unsaturated C_{18} - C_{22} -alkylcarbonyl; especially advantageously, are independently of one another C_{18} -, C_{20} - or C_{22} -alkylcarbonyl with at least two double bonds, advantageously with at least three, four, five or six double bonds, especially advantageously with at least four, five or six double bonds.

In a preferred embodiment of the process, a nucleic acid sequence which encodes polypeptides with ω 3-desaturase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105, or
- b) nucleic acid sequences which can be derived form the amino acid sequence shown in SEQ ID NO: 88 or SEQ ID NO: 106 as the result of the degeneracy of the genetic code, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105, which encode polypeptides with at least 60% identity at the amino acid level with SEQ ID NO: 88 or SEQ ID NO: 106 and which have ω 3-desaturase activity
- is additionally introduced into the transgenic plant.
- In a further preferred embodiment of the process, that a nucleic acid sequence which encodes polypeptides with $\Delta 12$ -desaturase activity, selected from the group consisting of:
- a) a nucleic acid sequence with the sequence shown in SEQ ₃₅ ID NO: 107, SEQ ID NO: 109 or SEQ ID NO: 195, or
- b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 108, SEQ ID NO: 110 or SEQ ID NO: 196, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 107, SEQ ID NO: 109 or SEQ ID NO: 195, which encode polypeptides with at least 60% at the amino acid level with SEQ ID NO: 108, SEQ ID NO: 110 or SEQ ID NO: 196 and which have Δ 12-desaturase activity is additionally introduced into the transgenic plant.

These abovementioned $\Delta 12$ -desaturase sequences can be used alone or in combination with $\omega 3$ -desaturase sequences together with the nucleic acid sequences used in the process which encode $\Delta 9$ -elongases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\Delta 5$ -elongases or $\Delta 4$ -desaturases.

Table 1 shows the nucleic acid sequences, the organism of origin and the sequence ID number.

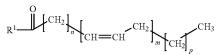
				-
No.	Organism	Activity	Sequence number	_
4.	Euglena gracilis Isochrysis galbana Phaeodactylum tricornutum Ceratodon purpureus Physcomitrella patens	Δ8-Desaturase Δ9-Elongase Δ5-Desaturase Δ5-Desaturase Δ5-Desaturase	SEQ ID NO: 1 SEQ ID NO: 3 SEQ ID NO: 5 SEQ ID NO: 7 SEQ ID NO: 9	• 60
6. 7. 8. 9. 10.	Thraustrochytrium sp. Mortierella alpina Caenorhabditis elegans Borago officinalis	Δ 5-Desaturase Δ 5-Desaturase Δ 5-Desaturase Δ 6-Desaturase Δ 6-Desaturase Δ 6-Desaturase	SEQ ID NO: 11 SEQ ID NO: 13 SEQ ID NO: 15 SEQ ID NO: 17 SEQ ID NO: 19 SEQ ID NO: 21	65

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-continue	20
-comunu	u

No.	Organism	Activity	Sequence number
12.	Physcomitrella patens	$\Delta 6$ -Desaturase	SEQ ID NO: 23
13.	Caenorhabditis elegans	$\Delta 6$ -Desaturase	SEQ ID NO: 25
14.	Physcomitrella patens	Δ 6-Elongase	SEQ ID NO: 27
15.	Thraustrochytrium sp.	$\Delta 6$ -Elongase	SEQ ID NO: 29
16.	Phytophtora infestans	$\Delta 6$ -Elongase	SEQ ID NO: 31
17.	Mortierella alpina	$\Delta 6$ -Elongase	SEQ ID NO: 33
18.	Mortierella alpina	$\Delta 6$ -Elongase	SEQ ID NO: 35
19.	Caenorhabditis elegans	$\Delta 6$ -Elongase	SEQ ID NO: 37
20.	Euglena gracilis	∆4-Desaturase	SEQ ID NO: 39
21.	Thraustrochytrium sp.	$\Delta 4$ -Desaturase	SEQ ID NO: 41
22.	Thalassiosira pseudonana	$\Delta 5$ -Elongase	SEQ ID NO: 43
23.	Thalassiosira pseudonana	∆6-Elongase	SEQ ID NO: 45
24.	Crypthecodinium cohnii	∆5-Elongase	SEQ ID NO: 47
25.	Crypthecodinium cohnii	Δ5-Elongase	SEQ ID NO: 49
26.	Oncorhynchus mykiss	∆5-Elongase	SEQ ID NO: 51
27.	Oncorhynchus mykiss	∆5-Elongase	SEQ ID NO: 53
28.	Thalassiosira pseudonana	∆5-Elongase	SEQ ID NO: 59
29.	Thalassiosira pseudonana	∆5-Elongase	SEQ ID NO: 61
30.	Thalassiosira pseudonana	∆5-Elongase	SEQ ID NO: 63
31.	Thraustrochytrium aureum	∆5-Elongase	SEQ ID NO: 65
32.	Ostreococcus tauri	$\Delta 5$ -Elongase	SEQ ID NO: 67
33.	Ostreococcus tauri	∆6-Elongase	SEQ ID NO: 69
34.	Primula farinosa	$\Delta 6$ -Desaturase	SEQ ID NO: 71
35.	Primula vialii	∆6-Desaturase	SEQ ID NO: 73
36. 37.	Ostreococcus tauri	∆5-Elongase	SEQ ID NO: 75
	Ostreococcus tauri	∆5-Elongase	SEQ ID NO: 77
38. 39.	Ostreococcus tauri	$\Delta 5$ -Elongase	SEQ ID NO: 79 SEQ ID NO: 81
39. 40.	Ostreococcus tauri Thraustrochytrium sp.	Δ6-Elongase Δ5-Elongase	SEQ ID NO: 81 SEQ ID NO: 83
40. 41.		$\Delta 5$ -Elongase	SEQ ID NO: 85 SEQ ID NO: 85
42.	Thalassiosira pseudonana Phytophtora infestans	ω3-Desaturase	SEQ ID NO: 85 SEQ ID NO: 87
42. 43.	Ostreococcus tauri	$\Delta 6$ -Desaturase	SEQ ID NO: 87 SEQ ID NO: 89
44.	Ostreococcus tauri	$\Delta 5$ -Desaturase	SEQ ID NO: 91
45.	Ostreococcus tauri	$\Delta 5$ -Desaturase	SEQ ID NO: 93
46.	Ostreococcus tauri	$\Delta 4$ -Desaturase	SEQ ID NO: 95
47.	Thalassiosira pseudonana	$\Delta 6$ -Desaturase	SEQ ID NO: 97
48.	Thalassiosira pseudonana	$\Delta 5$ -Desaturase	SEQ ID NO: 99
49.	Thalassiosira pseudonana	$\Delta 5$ -Desaturase	SEQ ID NO: 101
50.	Thalassiosira pseudonana	$\Delta 4$ -Desaturase	SEQ ID NO: 101 SEQ ID NO: 103
51.	Thalassiosira pseudonana	ω3-Desaturase	SEQ ID NO: 105
52.	Ostreococcus tauri	$\Delta 12$ -Desaturase	SEQ ID NO: 107
53.	Thalassiosira pseudonana	$\Delta 12$ -Desaturase	SEQ ID NO: 109
54.	Ostreococcus tauri	$\Delta 6$ -Elongase	SEQ ID NO: 111
55.	Ostreococcus tauri	$\Delta 5$ -Elongase	SEQ ID NO: 113
56.	Xenopus laevis (BC044967)	Δ5-Elongase	SEQ ID NO: 117
57.	Ciona intestinalis (AK112719)	$\Delta 5$ -Elongase	SEQ ID NO: 119
58.	Euglena gracilis	∆5-Elongase	SEQ ID NO: 131
59.	Euglena gracilis	$\Delta 5$ -Elongase	SEQ ID NO: 133
60.	Arabidopsis thaliana	Δ5-Elongase	SEQ ID NO: 135 SEQ ID NO: 135
61.	Arabidopsis thaliana	$\Delta 5$ -Elongase	SEQ ID NO: 137
62.	Phaeodactylum tricornutum	$\Delta 6$ -Elongase	SEQ ID NO: 183
63.	Phytium irregulare	$\Delta 6$ -Desaturase	SEQ ID NO: 193
64.	Calendula officinalis	$\Delta 12$ -Desaturase	SEQ ID NO: 195 SEQ ID NO: 195
65.	Ostreococcus tauri	$\Delta 5$ -Elongase	SEQ ID NO: 197
66.	Ostreococcus tauri	$\Delta 6$ -Elongase	SEQ ID NO: 199
67.	Ostreococcus tauri	$\Delta 6$ -Desaturase	SEQ ID NO: 201
07.	551,5550000 mm r	Lo Desaturdo	52Q ID 110.201

In a further embodiment of the invention, a process to be developed for the production of large amounts of polyunsaturated fatty acids, specifically ARA and EPA, in a transgenic plant. This process is also suitable for the production of DHA. Thus, ARA, EPA, DHA or their mixtures can be produced in the process. A further embodiment of the invention is thus a process for the compounds of the general formula I



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in transgenic plants, the process comprising:

- a) introducing, into a plant, at least one nucleic acid sequence which encodes a polypeptide with a Δ6-desaturase activity and is selected from the group consisting of:
 i) a nucleic acid with the sequence shown in SEQ ID NO: ⁵ 193 or SEO ID NO: 201.
 - ii) nucleic acid sequences which encode the amino acid sequence shown in SEQ ID NO: 194 or SEQ ID NO: 202,
 - iii) nucleic acid sequences which hybridize under stringent conditions with the complementary strand of the nucleic acid sequence shown in SEQ ID NO: 193 or SEQ ID NO: 201, and
 - iv) nucleic acid sequences which have at least 60% 15 identity with the sequence shown in SEQ ID NO: 193 or SEQ ID NO: 201,
- b) introducing, into a plant, at least one nucleic acid sequence which encodes a polypeptide with a Δ6-elon-gase activity and is selected from the group consisting of: 20
 i) a nucleic acid with the sequence shown in SEQ ID NO:
 - 27 or SEQ ID NO: 199, ii) nucleic acid sequences which encode the amino acid
 - sequence shown in SEQ ID NO: 28 or SEQ ID NO: 200,
 - iii) nucleic acid sequences which hybridize under stringent conditions with the complementary strand of the nucleic acid sequence shown in SEQ ID NO: 27 or SEQ ID NO: 199, and
 - iv) nucleic acid sequences which have at least 60% identity with the sequence shown in SEQ ID NO: 27 or SEQ ID NO: 199,
- c) introducing, into a plant, at least one nucleic acid sequence which encodes a polypeptide with a Δ5-desaturase activity and is selected from the group consisting of:
 i) a nucleic acid with the sequence shown in SEQ ID NO: 11.
 - ii) nucleic acid sequences which encode the amino acid sequence shown in SEQ ID NO: 12,
 - iii) nucleic acid sequences which hybridize under stringent conditions with the complementary strand of the nucleic acid sequence shown in SEQ ID NO: 11, and
- iv) nucleic acid sequences which have at least 60% identity with the sequence shown in SEQ ID NO: 11, 45 where the variables and substituents in the formula I have the meaning given above.

The nucleic acid sequences which can be used in the process are described in WO 02/26946 (Δ 5-desaturase from *Thraustochytrium* ssp., SEQ ID NO: 11 and Δ 6-desaturase 50 from *Phytium irregulare*, SEQ ID NO: 193) and in WO 01/59128 (Δ 6-elongase from *Physcomitrella patens*, SEQ ID NO: 27), which is expressly referred to here. However, in these cases, the formation of ARA and EPA was studied either not in transgenic plants, but only in microorganisms, 55 or else no increase ARA and EPA synthesis was detected in the transgenic plants. Moreover, the nucleic acids according to the invention were not combined, in these applications, with nucleic acids which encode other enzymes of the fatty acid biosynthetic pathway.

Surprisingly, it has now been found that the coexpression of the nucleic acids with the sequences shown in SEQ ID NO: 11, 27, 193, 199 and 201 leads, in transgenic plants, to a greatly increased ARA content to up to more than 8%, advantageously up to more than 10%, 11%, 12%, 13%, 14%, 65 15%, 16%, 17%, 18%, 19% or 20%, especially advantageously to more than 21%, 22%, 23%, 24% or 25%, based

on the total lipid content of the plant (cf. Table 2, Table 3, Table 4 and FIG. **31**). The abovementioned percentages are percent by weight.

To further increase the yields in the process described for the production of oils and/or triglycerides with a content of polyunsaturated fatty acids, especially ARA, EPA or DHA or their mixtures, which is advantageously increased in comparison with oils and/or triglycerides from wild-type plants, it may be advantageous to increase the amount of the starting material for the fatty acid biosynthesis. This can be achieved for example by introducing a nucleic acid which encodes a polypeptide with the activity of a $\Delta 12$ -desaturase, and coexpressing it in the organism.

This is especially advantageously in oil-producing organisms such as the family Brassicaceae, such as the genus *Brassica*, for example oilseed rape, turnip rape or Indian mustard; the family Elaeagnaceae, such as the genus *Elaeagnus*, for the 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 has a high oleic acid content, but only a low linoleic acid content (Mikoklajczak et al., Journal of the American Oil Chemical Society, 38, 1961, 678-681).

This is why, in a preferred embodiment of the present invention, a nucleic acid sequence which encodes a polypeptide with Δ 12-desaturase activity is additionally introduced into the transgenic plant.

Especially preferably, this nucleic acid sequence is selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 195,
- b) nucleic acid sequences which encode the amino acid sequence shown in SEQ ID NO: 196,
- c) nucleic acid sequences which hybridize under stringent conditions with the complementary strand of the nucleic acid sequence shown in SEQ ID NO: 195, and
- d) nucleic acid sequences which have at least 60% identity with the sequence shown in SEQ ID NO: 195.

The nucleic acid sequence with the SEQ ID NO: 195 is derived from *Calendula officinalis* and described in WO 01/85968, the disclosure of which is likewise incorporated in the present application by reference.

The $\Delta 12$ -desaturases used in the process according to the invention advantageously convert oleic acid (C18:1^{$\Delta 9$}) into linoleic acid (C18:2^{$\Delta 9$,12}) or C18:2^{$\Delta 6,9$} into C18:3^{$\Delta 6,9,12$} (gamma-linolenic acid=GLA), the starting materials for the synthesis of ARA, EPA and DHA. The $\Delta 12$ -desaturases advantageously convert fatty acids bound to phospholipids or CoA-fatty acid esters, advantageously bound to CoA-fatty acid esters. If an elongation step has taken place beforehand, this advantageously leads to higher yields of synthetic products since, as a rule, elongation takes place at CoA-fatty acid esters, while desaturation predominantly takes place at the phospholipid or at the triglycerides. An exchange between the CoA-fatty acid esters and the phospholipids or triglycerides, which would require a further, potentially limiting, enzyme reaction, is thus not required.

The additional expression of the $\Delta 12$ -desaturase in the transgenic plants leads to a further increase in the ARA content up to more than 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% and 20%, especially advantageously to more than 21%, 22%, 23%, 24% or 25%, based on the total lipid content of the plant (cf. Tables 3 and 4 and FIG. **32**). The abovementioned percentages are percent by weight.

Further nucleic acid sequences which encode a polypeptide with a $\Delta 5$ -elongase activity can advantageously be introduced into the plants in the process according to the invention.

Preference is given to those nucleic acid sequences which 5 encode a Δ 5-elongase activity is chosen from the group consisting of:

- a) a nucleic acid sequence was the sequence shown in SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 6.5, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 197,
- b) nucleic acid sequences which encode the amino acid sequence shown in SEQ ID NO: 44, SEQ ID NO: 48, SEQ iD NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID 20
 iD NO: 50, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO; 86, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID 25
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- c) nucleic acid sequences which hybridize under stringent conditions with the complementary strand of the nucleic acid sequence shown, in SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ 30 ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, 35 SEQ ID NO: 137 or SEQ ID NO: 197, and
- d) nucleic acid sequences which have at least 60% identity with the sequence shown in SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO:49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ 40 ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 197.

In a preferred embodiment of the process, the $\Delta 5$ -elongase genes are expressed under the control of a seed-specific promoter.

In a further advantageous embodiment of the process, all nucleic acid sequences are introduced into the plants on a 50 shared recombinant nucleic acid molecule, it being possible for each nucleic acid sequence to be under the control of its own promoter and it being possible for this own promoter to take the form of a seed-specific promoter.

However, it is not only the nucleic acids detailed in the 55 sequence listing which can successfully be employed in the invention to carry out the conversion; rather, even sequences which deviate to a certain degree from these sequences and which encode proteins with the essentially identical enzymatic activity can be employed. These take the form of 60 nucleic acids which have a certain degree of identity or homology with the sequences specified in the sequence listing. An essentially identical enzymatic activity denotes proteins which have at least 20%, 30%, 40%, 50% or 60%, advantageously at least 70%, 80%, 90% or 95%, especially 65 advantageously at least 96%, 97%, 98% or 99% of the enzymatic activity of the wild-type enzymes.

In order to determine the percentage of homology (=identity) of two amino acid sequences or of two nucleic acids, the sequences are written one under the other (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 the other 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 positions which the sequences share (i.e. % homology=number of identical positions/total number of positions×100). The terms homology and identity are therefore to be considered as synony-

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., 25, 351-360, 1987, Higgins et al., CABIOS, 51989:151-153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wis., USA 53711 (1991)], were used to carry out the sequence comparisons. The sequence homology data given above in percent 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.

The skilled worker will recognize that DNA sequence polymorphisms which lead to modifications of the amino acid sequence of SEQ ID NO: 12, 28, 194, 196, 198, 200 and/or 202 may occur within a population. These natural variants usually cause a variance of from 1 to 5% in the nucleotide sequence of the Δ 12-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase and/or Δ 6-elongase gene. The scope of the invention is to comprise each and all of these nucleotide variation(s) and resulting amino acid polymorphisms in the Δ 12-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase and/or Δ 6-elongase which are the result of natural variation and which do not essentially modify the enzymatic activity.

Essential enzymatic activity of the $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -elongase or $\Delta 5$ -desaturase used in the process according to the invention is understood as meaning that they retain an enzymatic activity of at least 10%, preferably of at least 20%, especially preferably of at least 30%, 40%, 50% or at least 60% and most preferably at least 70%; 80%, 90%, 95%, 96%, 97%, 98% or 99% in comparison with the proteins/enzymes encoded by the sequence and its derivatives and that they are thus capable of participating in the metabolism of compounds which are required for the synthesis of fatty acids, fatty acid esters such as diacylglycerides and/or triacylglycerides in a plant or plant cell or in the transport of molecules across membranes,

meaning C_{18} -, C_{20} - or C_{22} -carbon chains in the fatty acid molecule with double bonds at least two, advantageously three, four or five, positions.

Likewise, the scope of the invention comprises nucleic acid molecules which hybridize under stringent conditions 5 with the complementary strand of the $\Delta 12$ -desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase and/or Δ 6-elongase nucleic acids used. The term "hybridizes under stringent conditions" as used in the present context is to describe hybridization and washing conditions under which nucleo-10 tide sequences with at least 60% homology to one another usually remain hybridized with one another. Conditions are preferably such that sequences with at least approximately 65%, 70%, 80% or 90%, preferably at least approximately 91%, 92%, 93%, 94% or 95%, and especially preferably at 15 least approximately 96%, 97%, 98%, 99% or more homology to one another usually remain hybridized to one another. These stringent conditions are known to the skilled worker and described, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. 20

A preferred, nonlimiting, example of stringent hybridization conditions is hybridizations in 6× sodium chloride/ sodium citrate (=SSC) at approximately 45° C., followed by one or more washing steps in 0.2×SSC, 0.1% SDS at 50 to 65° C. The skilled worker knows that these hybridization 25 conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, regarding temperature and buffer concentration. Under "standard hybridization conditions", for example, the hybridization temperature is, depending on the type of nucleic acid, 30 between 42° C. and 58° C. in aqueous buffer with a concentration of 0.1 to 5×SSC (pH 7.2). If organic solvents, for example 50% formamide, are present in the abovementioned buffer, the temperature under standard conditions is approximately 42° C. Preferably the hybridization condi- 35 tions for DNA:DNA hybrids, for example, are 0.1×SSC and 20° C. to 45° C., preferably 30° C. to 45° C. Preferably the hybridization conditions for DNA:RNA hybrids are, for example, 0.1×SSC and 30° C. to 55° C., preferably 45° C. to 55° C. The abovementioned hybridization temperatures 40 are determined for a nucleic acid with approximately 100 bp (=base pairs) in length and with a G+C content of 50% in the absence of formamide. The skilled worker knows how to determine the required hybridization conditions on the basis of textbooks such as Sambrook et al., "Molecular Cloning", 45 Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Eds.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, 50 Oxford.

By introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence, it is possible to generate an isolated nucleic acid molecule which encodes a Δ 12-desaturase, Δ 6-desaturase, Δ 5-desaturase, 55 Δ 5-elongase and/or Δ 6-elongase with one or more amino acid substitutions, additions or deletions. Mutations can be introduced into one of the sequences by means of standard techniques, such as site-specific mutagenesis and PCRmediated mutagenesis. It is preferred to generate conserva- 60 tive amino acid substitutions in one or more of the above nonessential amino acid radicals. In a "conservative amino acid substitution", the amino acid radical is replaced by an amino acid radical with a similar side chain. Families of amino acid radicals with similar side chains have been 65 defined in the art. These families comprise amino acids with basic side chains (for example lysine, arginine, histidine),

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acidic side chains (for example aspartic acid, glutamic acid), uncharged polar side chains (for example glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), unpolar side chains (for example alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), betabranched side chains (for example threonine, valine, isoleucine) and aromatic side chains (for example tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid radical in a Δ 12-desaturase, Δ 6-desaturase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 6$ -elongase is thus preferably replaced by another amino acid radical from the same family of side chains. In another embodiment, the mutations can, alternatively, be introduced randomly over all or part of the sequence encoding the $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, Δ 5-desaturase, Δ 5-elongase or Δ 6-elongase, for example by saturation mutagenesis, and the resulting mutants can be screened by recombinant expression for the hereindescribed Δ 12-desaturase, Δ 6-desaturase, Δ 5-desaturase, Δ 5-elongase or $\Delta 6$ -elongase activity in order to identify mutants which have retained the $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 6$ -elongase activity.

The polyunsaturated fatty acids produced in the process according to the invention advantageously comprise at least two, preferably three, four, five or six, double bonds. The fatty acids especially preferably comprise four, five or six double bonds. Fatty acids produced in the process preferably have a length of 20 C or 22 C atoms.

Saturated fatty acids are preferably reacted to a minor degree with the nucleic acids used in the process, or not at all. "A minor degree" is understood as meaning that, in comparison with polyunsaturated fatty acids, the saturated fatty acids are reacted with less than 5%, preferably with less than 3%, especially preferably with less than 2%, most preferably with less than 1, 0.5, 0.25 or 0.125% of the activity. The fatty acids produced may constitute the only product of the process or else may be present in a fatty acid mixture.

The polyunsaturated fatty acids produced in the process are advantageously bound in membrane lipids and/or triacylglycerides, but may also occur in the organisms as free fatty acids or else bound in the form of other fatty acid esters. In this context, they may be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different glycerides. The various fatty acids which are bound in the triacylglycerides can be derived from short-chain fatty acids with 4 to 6 C atoms, medium-chain fatty acids with 8 to 12 C atoms or long-chain fatty acids with 14 to 24 C atoms, preferred are the long-chain fatty acids, especially preferred are the longchain fatty acids LCPUFAs of C18-, C20- and/or C22-fatty acids, very especially preferred are the long-chain fatty acids LCPUFAs of C_{20} - and/or C_{22} -fatty acids such as ARA, EPA, DHA or their combination.

The process according to the invention advantageously yields fatty acid esters with polyunsaturated C_{18} -, C_{20} - and/or C_{22} -fatty acid molecules with at least two double bonds in the fatty acid ester, advantageously with at least three, four, five or six double bonds in the fatty acid ester, especially advantageously four, five or six double bonds in the fatty acid ester, especially advantageously four, five or six double bonds in the fatty acid ester, especially advantageously four, five or six double bonds in the fatty acid ester, respecially advantageously at least five or six double bonds in the fatty acid ester. This advantageously leads to the synthesis of linoleic acid (=LA, C18:2^{\Delta_{9},12}), \gamma-linolenic acid (=GLA, C18:3^{\Delta_{6},9,12}), stearidonic acid (=SDA, C18:4^{\Delta_{6},9,12,15}), dihomo- γ -linolenic acid (=ETA, C20:4^{\Delta_{5},8,11,14}), arachidonic acid (ARA, C20:4^{\Delta_{5},8,11,14}), eicosapentaenoic acid (EPA, C20:4^{\Delta_{5},8,11,14}) or mixtures of

these, ω 3-eicosapentaenoic acid (=ETA, C20:4^{Δ 5,8,11,14,17}), arachidonic acid (ARA, C20:4^{Δ 5,8,11,14}), eicosapentaenoic acid (EPA, C20:5^{Δ 5,8,11,14,17})) ω 6-docosapentaenoic acid (C22:5^{Δ 4,7,10,13,16}), ω 6-docosapentaenoic acid (C22:4^{Δ 7,10,13,16}), ω 3-docosapentaenoic acid (=DPA, C22: 5 5^{Δ 7,10,13,16,19}), docosahexaenoic acid (=DHA, C22:6^{Δ 4,7,10,13,16,19}) or their mixtures are preferably produced, and ARA, EPA and/or DHA are very especially produced. ω 3-Fatty acids such as EPA and/or DHA, preferably DHA, are advantageously produced.

The fatty acid esters with polyunsaturated C18-, C20and/or C22-fatty acid molecules, advantageously with polyunsaturated-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, 15 lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters 20 such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three, four, five or six, preferably four, five or six, especially preferably five or six, double bonds, from the plants which were used for the preparation of the fatty acid esters. Preferably, they are 25 isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of phosphatidylcholine, especially preferably in the form of the triacylglycerides. In addition to these esters, the polyunsaturated fatty acids are also present in the plants as free fatty acids or bound in other compounds. 30 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 35 acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

In the method(s) according to the invention (for the purposes of the invention and the disclosure shown herein, the singular is to comprise the plural and vice versa), the 40 LCPUFAs produced are produced in a content of at least 3, 5, 6, 7 or 8% by weight, advantageously at least 9, 10, 11, 12, 13, 14 or 15% by weight, preferably at least 16, 17, 18, 19 or 20% by weight, especially preferably at least 21, 22, 23, 24 or 25% by weight, very especially preferably at least 45 26, 27, 28, 29 or 30% by weight based on the total fatty acids in the transgenic organisms, advantageously in the seeds of the transgenic plants. Here, C18- and/or C20-fatty acids which are present in the host organisms are advantageously converted into the corresponding products such as ARA, 50 EPA, DPA or DHA, to mention but a few by way of example, at the rate of at least 10%, advantageously at least 20%, especially advantageously at least 30%, very especially advantageously at least 40%. The fatty acids are advantageously produced in bound form.

Polyunsaturated C_{20} -fatty acids with four or five double bonds in the molecule are advantageously produced in the process in a content of all such fatty acids together of at least 15, 16, 17, 18, 19, or 20% by weight, advantageously at least 21, 22, 23, 24 or 25% by weight, especially advantageously ⁶⁰ at least 26, 27, 28, 29 or 30% by weight based on the total fatty acids, in the seeds of the transgenic plants.

Polyunsaturated C_{20} - and/or C_{22} -fatty acids with four, five or six double bonds in the molecule are advantageously produced in the process in a content of all such fatty acids 65 together of at least 15, 16, 17, 18, 19, or 20% by weight, advantageously at least 21, 22, 23, 24 or 25% by weight,

especially advantageously at least 26, 27; 28, 29 or 30% by weight, very especially advantageously at least 31, 32, 33, 34 or 35% by weight based on the total fatty acids in the seeds of the transgenic plants.

ARA is produced in the process according to the invention in a content of at least 3, 5, 6, 7, 8, 9 or 10% by weight, advantageously at least 11, 12, 13, 14 or 15% by weight, preferably at least 16, 17, 18, 19 or 20% by weight, especially preferably at least 21, 22, 23, 24 or 25% by weight, most preferably at least 26% by weight, based on the total lipid content in the seeds of the transgenic plants.

EPA is produced in the process according to the invention in a content of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% by weight, advantageously at least 2, 3, 4 or 5% by weight, preferably at least 6, 7, 8, 9 or 10% by weight, especially preferably at least 11, 12, 13, 14 or 15% by weight and most preferably at least 16% by weight, based on the total lipid content in the seeds of transgenic plants.

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.

It is possible, with the aid of the nucleic acids used in the process according to the invention, for these unsaturated fatty acids to be positioned at the sn1, sn2 and/or sn3 position of the triglycerides which have advantageously been produced. Since in the process according to the invention the starting compounds linoleic acid (C18:2) and linolenic acid (C18:3) pass through a plurality of reaction steps, the end product of the process, such as, for example, arachidonic acid (ARA), eicosapentaenoic acid (EPA), ω6-docosapentaenoic acid or DHA, are not obtained as absolutely pure products, small traces of the precursors are also always present in the end product. If, for example, both linoleic acid and linolenic acid are present in the starting organism, or the starting plants, the end product, such as ARA, EPA or DHA, are present as mixtures. It is advantageous that, in the end product ARA or DHA, only minor amounts of the in each case other end product should be present. This is why, in a DHA-comprising lipid and/or oil, less than 15, 14, 13, 12 or 11% by weight, advantageously less than 10, 9, 8, 7, 6 or 5% by weight, especially advantageously less than 4, 3, 2 or 1% by weight, of EPA and/or ARA should be present. This is why, in a EPA-comprising lipid and/or oil, less than 15, 14, 13, 12 or 11% by weight, advantageously less than 10, 9, 8, 7, 6 or 5% by weight, especially advantageously less than 4, 3, 2 or 1% by weight, of ARA should be present. This is also why less than 15, 14, 13, 12 or 11% by weight, advantageously less than 10, 9, 8, 7, 6 or 5% by weight, especially advantageously less than 4, 3, 2 or 1% by weight of EPA and/or DHA should be present 55 in an ARA-comprising lipid and/or oil.

However, mixtures of different polyunsaturated C_{20} - and/ or C_{22} -fatty acids in one product may also be desirable. In such cases, DHA-comprising lipids and/or oils may comprise at least 1, 2, 3, 4 or 5% by weight of ARA and/or EPA, advantageously at least 6, 7 or 8% by weight, especially advantageously at least 9, 10, 11, 12, 13, 14 or 15% by weight, very especially advantageously at least 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25% by weight, based on the total lipid content in the seeds of the transgenic plants.

The precursors should advantageously not amount to more than 20% by weight, preferably not to more than 15% by weight, especially preferably not to more than 10% by

weight, very especially preferably not to more than 5% by weight, based on the amount of the end product in question. Advantageously, only ARA, EPA or only DHA, bound or as free acids, are produced as end products in the process of the invention in a transgenic plant. If the compounds ARA, EPA 5 and DHA are produced simultaneously, they are advantageously produced in a ratio of at least 1:1:2 (EPA:ARA: DHA), advantageously at least 1:1:3, preferably 1:1:4, especially preferably 1:1:5. If the compounds ARA and EPA are produced simultaneously, they are advantageously pro-10 duced, in the plant, in a ratio of at least 1:6 (EPA:ARA), advantageously of at least 1:8, preferably of at least 1:10, especially preferably of at least 1:12.

Fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise 15 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total 20 fatty acid content of the organisms.

Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention advantageously comprise fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic 25 acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid, chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic 30 acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic 35 acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13coctadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid 40 (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (allcis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The abovementioned fatty acids are, as a rule, advan- 45 tageously only found in traces in the fatty acid esters or fatty acid mixtures produced by the process according to the invention, that is to say that, based on the total fatty acids, they occur to less than 30%, preferably to less than 25%, 24%, 23%, 22% or 21%, especially preferably to less than 50 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very especially preferably to less than 4%, 3%, 2% or 1%. In a further preferred form of the invention, these abovementioned fatty acids occur to less than 0.9%, 0.8%, 0.7%, 0.6% or 0.5%, especially preferably to less than 0.4%, 0.3%, 0.2%, 0.1%, 55 based on the total fatty acids. 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 butyric acid, no cholesterol, no clupanodonic acid (=docosapentaenoic acid, $C22:5^{\Delta4,8,12,15,21}$) and no nisinic acid (tetracosahexaenoic acid, 60 acid, C23:6 $^{\Delta 3,8,12,15,18,21}$).

Owing to the nucleic acid sequences according to the invention or nucleic acid sequences used in the process according to the invention, an increase in the yield of 65 polyunsaturated fatty acids, mainly ARA and EPA, but also DHA, of at least 50, 80 or 100%, advantageously at least

150, 200 or 250%, especially advantageously at least 300, 400, 500, 600, 700, 800 or 900%, very especially advantageously at least 1000, 1100, 1200, 1300, 1400 or 1500% in comparison with the nontransgenic starting plant, for example a plant such as *Brassica juncea, Brassica napus, Camelina sativa, Arabidopsis thanliana* or *Linum usitatissimum* when compared by means of GC analysis; see Examples.

Advantageously, as described above, the polyunsaturated C20- and/or C22-fatty acids with four, five or six double bonds in the molecule, which are produced in the process, will comprise in the seeds of plants which comprise only very small amounts of C12:0- or C14:0-fatty acids, or none at all. Even shorter saturated fatty acids, such as the fatty acids C4:0, C6:0, C8:0 or C10:0 should not be present in the lipid and/or oil or only in very small amounts. Only very small amounts are advantageously understood as amounts which, in GC analysis, are advantageously under 5, 4, 3, 2 or 1%, advantageously under 0.9, 0.8, 0.7, 0.6 or 0.5%, especially advantageously under 0.4, 0.3, 0.2 or 0.1%, very especially preferably under 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02 or 0.01 units area in the GC. The fatty acid C16:0 should advantageously be in a range of from 1 to 28% GC units area. The fatty acid C16:0 should advantageously be present in GC units area in amounts of less than 25%, 20%, 15% or 10%, advantageously less than 9%, 8%, 7%, 6% or 5%, especially advantageously less than 4%, 3%, 2% or 1% or not at all, in the lipids, oils and/or free fatty acids. The fatty acid C16:1 should advantageously amount to less than 1, 0.5, 0.4, 0.3, 0.2 or 0.1%, especially advantageously 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02 or 0.01 units area in the GC. Very especially preferably, the fatty acid C16:1 should not be present in the oils and/or lipids produced by the process. The same applies to the fatty acids C15:0, C17:0, C16:1^{Δ 3} trans, C16:4^{Δ 4,7,10,13} and C18:5^{Δ 3,6,9,12,15}. Besides oleic acid (C18:1^{Δ 9}), the isomers (C18:1^{Δ 7}, 18:1^{Δ 11}) may also be present in the lipids, oils or free fatty acids. Advantageously in amounts of less than 5%, 4%, 3%, 2% or 1%, measured as units GC area. The fatty acids C20:0, C20:1, C24:0 and C24:1 should in each case be in the range of from 0 to 1%, 0 to 3% and 0 to 5%, respectively, units GC area. Furthermore, little dihomo-y-linolenic acid (=DGLA) should be detectable in the GC analysis in units GC area in the seed oil and/or seed lipid. Little is understood as meaning less than 2, 1.9, 1,8, 1.7, 1.6 or 1.5%, advantageously less than 1.4, 1.3, 1.2, 1.1 or 1%, especially advantageously less than 0.9, 0.8, 0.7, 0.6, 0.5 or 0.4% in units GC area.

In a preferred embodiment of the process, DGLA and ARA should be produced in a ratio of from 1:1 up to 1:100, advantageously from 1:2 up to 1:80, especially advantageously from 1:3 up to 1:70, very especially from 1:5 up to 1:60.

In a further preferred embodiment, DGLA and EPA should be produced in a ratio of from 1:1 up to 1:100, advantageously from 1:2 up to 1:80, especially advantageously from 1:3 up to 1:70, very especially from 1:5 up to 1:60.

The lipids and/or oils produced in the process according to the invention should advantageously have a high unsaturated, advantageously polyunsaturated, fatty acid content of at least 30, 40 or 50% by weight, advantageously at least 60, 70 or 80% by weight, based on the total fatty acid content in the seeds of the transgenic plants.

All saturated fatty acids together should advantageously only amount to a small quantity in the plants preferably used in the process according to the invention. In this context, a small amount is understood as meaning an amount of less than 15%, 14%, 13%, 12%, 11% or 10%, preferably less than 9%, 8%, 7% or 6%, in units GC area.

Furthermore, the genes for the synthesis of the polyunsaturated fatty acids, which are used in the process and which have been introduced, in the process, via different processes, advantageously as host plant, should advantageously have a higher oil content than protein content in the seed, advantageous plants have an oil/protein content ratio of from 5:1, 4:1, 3:1, 2:1 or 1:1. In this context, the oil content based on the total weight of the seed should be in a range of 15-55%, advantageously between 25-50%, especially advantageously between 35-50%. Advantageous host plants used in the process should have a distribution of the unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid, which are the starting compounds in the process according to the invention for the synthesis of polyunsaturated fatty acids, in the sn1, sn2 and sn3 position of the triglyceride, as shown in Table 5 hereinbelow, where rows No. 1-7 represent different advantageous alternatives 20 of such distributions, n.p. means not present.

TABLE 5

Plants with advantageous fatty acid distribution in the sn1, sn2 and sn3 position on the triglyceride							25			
	Oleic acid		Linoleic acid		α-Linolenic acid		_			
No.	sn1	sn2	sn3	sn1	sn2	sn3	sn1	sn2	sn3	
1.	1	1	1	2	4	1	n.p.	n.p.	n.p.	. 30
2.	1.4	2.2	1	2.8	9	1	2	6.7	1	
3.	0.8	0.8	1	1.1	1.6	1	1	0.8	1	
4.	0.9	0.9	1	1.2	1.6	1	0.9	1	1	
5.	0.9	0.9	1	1	1.3	1	1	1	1	
6.	1	1.1	1	2	2.8	1	1	1	n.p.	
7.	1.3	9.7	1	1	9	traces	1	n.p.	n.p.	35

The rows show the ratios of the following plants: row 1=Arachis hypogaea, row 2=Brassica napus, row 3=Glycine max, row 4=Linum usitatissimum, row 5=Zea mays, row 6=Olea europaea and row 7=Theobroma cacao.

Host plants which are advantageous for the process are those which have a high oleic acid content, that means at least 40, 50, 60 or 70% by weight based on the total fatty acid content of the plant, in comparison with linoleic acid and/or linolenic acid in the lipids and/or oils, especially in 45 the triglyceride, such as, for example, Anarcardium occidentale, Argania spinosa, Bombax malabaricum, Brassica napus, Butyrospermum parkii, high-oleic safflower (Carthamus tinctorius), Citrullus colocythis, Corylus avellana, Curcurbita foetidissima, Curcurbita pepo, Guizotia abyssinica, 50 high-oleic sunflower (Helianthus annus), Macadamia intergrifolia, Nigella sativa, Olea europaea, Papaver somniferium, Passiflora edulis, Persea americana, Prunus amygdalis, Prunus armeniaca, Prunus dulcis, Prunus communis, Sesamum indicum, Simarouba glauca, Thea sasumgua, or 55 Theobroma cacao. Further advantageous plants have a higher content of the unsaturated fatty acids oleic acid, linoleic acid and α -linolenic acid in the sn2 position in comparison with the other positions sn1 and sn3. A higher content is understood as meaning ratios of (sn1:sn2:sn3) 60 1:1.1:1, 1:1.5:1 to 1:3:1. Advantageous plants such as Actinidia chinensis, Aleurites moluccana, Arnebia griffithii, Brassica alba, Brassica hirta, Brassica nigra, Brassica juncea, Brassica carinata, Camelina sativa, Cannabis sativa, Echium rubrum, Echium vulgare, Humulus lupulus, 65 Juglans regia, Linum usitatissimum, Ocimum spp., Perilla frutescens, Portulaca oleracea, Prunus cerasus, Salicornia

bigelovii, Salvia hispanica are also those which have a high α -linolenic acid content in the lipid and/or oil of the plant, that is to say an α -linolenic acid content of at least 10, 15 or 20% by weight, advantageously at least 25, 30, 35, 40, 45 or 50% by weight, based on the total fatty acid content of the plant. Very especially advantageous plants likewise show an advantageous preference for the sn2 position over the positions sn1 and sn3 in the triglyceride of from 1:1.1:1, 1:1.5:1 to 1:3:1 for the arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid produced in the process.

Plants used for the process should advantageously have an erucic acid content of less than 2% by weight based on the total fatty acid content of the plant. Also, the content of saturated fatty acids C16:0 and/or C18:0 should advantageously be less than 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10% by weight; advantageously less than 9, 8, 7, 6 or 5% by weight, based on the total fatty acid content of the plant. Also, longer fatty acids such as C20:0 or C22:1 should advantageously not be present, or only in small amounts, advantageously in amounts of less than 4, 3, 2 or 1% by weight, advantageously less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 or 0.1% by weight based on the total fatty acid content of the plant in the plants used in the process. Typically, C16:1 is not present as fatty acid, or only present in small amounts, in the plants used for the process according to the invention. Small amounts are advantageously understood as meaning fatty acid contents which are less than 4, 3, 2 or 1% by weight, advantageously less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 or 0.1% by weight based on 30 the total fatty acid content of the plant.

For economic reasons, that is to say because of the area under cultivation and the oil yield, plants which are grown on a large scale, such as soybean, oilseed rape, mustard, *Camelina*, linseed, sunflower, oil palm, cotton, sesame, maize, olive, are preferred, preferably oilseed rape, *Camelina*, linseed, sunflower are used frequently as host plant in the process.

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, advantageously the seeds of the plants, in the known manner, for example via crushing the seeds, such as grinding, followed by 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.

Plants which are suitable for the process according to the invention are, in principle, all those plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. Advantageous plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Compositae, Convolvulaceae, Cucurbitaceae, Elaeagnaceae, Ericaceae, Cruciferae. Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae, Olacaceae, Oleaceae, Papaveraceae, Piperaceae, Pedaliaceae, Poaceae, Rosaceae or Solanaceae, vorteilhaft Anacardiaceae, Asteraceae, Boraginaceae, Brassicaceae, Cannabaceae, Compositae, Cruciferae, Cucurbitaceae, Elaeagnaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Leguminosae; Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae, Olacaceae, Oleaceae, Papaveraceae, Piperaceae,

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Pedaliaceae, Poaceae or Solaneae, but other plants which are suitable for the process are vegetable plants or ornamentals such as *Tagetes*.

Examples which may be mentioned are the following plants selected from the group consisting of: Anacardiaceae 5 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, 10 Locusta, Tagetes, Valeriana, for example the genus and species Artemisia sphaerocephala, Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], 15 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 [salad vegetables], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia 20 [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus colurna [hazelnut], Boraginaceae, such as the genus Adelocarvum, 25 Alkanna, Anchusa, Borago, Brunnera, Cerinthe, Cynoglossum, Echium, Gastrocatyle, Lithospermum, Moltkia, Nonea, Onosma, Onosmodium, Paracaryum, Pectocarya, Symphytum for example the genus and species Adelocarym coelestinum, Alkanna orientalis, Anchusa anzurea, Anchusa capen- 30 sis, Anchusa hybrida, Borago officinalis [borage], Brunnera orientalis, Cerinthe minor, Cynoglossum amabile, Cynoglossum lanceolatum, Echium rubrum, Echium vulgare, Gastrocatyle hispida, Lithospermum arvense, Lithosperumum purpureocaeruleum, Mbltkia aurea, Moltkia coerules, 35 Nonea macrosperma, Onosma sericeum, Onosmodium molle, Onosmodium occidentale, Paracaryum caelestinum, Pectocarya platycarpa, Symphytum officinale, Brassicaceae, such as the genera Brassica, Camelina, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species 40 Brassica alba, Brassica carinata, Brassica hirta, 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, Melano- 45 sinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species Anana comosus, Ananas ananas or Bromelia comosa [pineapple], Caricaceae, such as the genus Carica, 50 such as the genus and species, Carica papaya [pawpaw], Cannabaceae, such as the genus *Cannabis*, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species Ipomoea batatus, Ipomoea pandurata, Convol- 55 vulus 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 60 maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the 65 genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash],

Elaeagnaceae, such as the genus *Elaeagnus*, for example the genus and species Olea europaea [olive], Ericaceae, such as the genus Kalmia, for example the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentals, Cistus chamaerhodendros or Kalmia lucida [mountain laurel], Euphorbiaceae, such as the genera Manihot, Janipha, Jatropha, Ricinus, for example 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, Medicajo, Glycine, Dolichos, Phaseolus, soybean, for example the genera and species Pisum sativum, Pisum arvense, Pisum humile [pea], Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbeck, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], 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, for example the genera and species Cocos nucifera, Pelargonium grossularioides or Oleum cocois [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra or Wallia nigra [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species Laurus nobilis [bay], Persea americana, Persea gratissima or Persea persea [avocado], Leguminosae, such as the genus Arachis, for example the genus and species Arachis hypogaea [peanut], Linaceae, such as the genera Adenolinum, for example 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 [linseed], Lythrarieae, such as the genus Punica, for example the genus and species Punica granatum [pomegranate], Malvaceae, such as the genus Gossvpium, for example the genera and species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species Marchantia berteroana, Marchantia foliacea, Marchantia macropora, Musaceae, such as the genus Musa, for example the genera and species Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species Oenothera biennis or Camissonia brevipes [evening primrose], Palmae, such as the genus *Elaeis*, for example the genus and species *Elaeis* guineensis [oil palm], Papaveraceae, such as, for example, the genus Papaver, for example the genera and species Papaver orientate, Papaver rhoeas, Papaver dubium

example the genus and species Sesamum indicum [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, 5 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, 10 Oryza, Zea (maize), Triticum, for example 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 15 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 aethio- 20 picum, 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, Hol- 25 cus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza iatifolia [rice], Zea mays [maize] Triticum aestivum, Triticum durum, Triticumt turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare [wheat]; Porphyridiaceae, such as the 30 genera Chrodthece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Rosaceae, such as 35 the genus Prunus, for example the genus and species Prunus armeriiaca, Prunus amygdalus, Prunus avilum, Rubiaceae, such as the genus Coffea, for example the genera and species Coffea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae, such as the genus Scro- 40 phularia, Verbascum, for example the genera and species Scrophularia marilandica, Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, 45 Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae, such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens [pepper], 50 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 melon- 55 gena [eggplant] Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solarium lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus 60 Camellia, for example the genus and species Camellia sinensis [tea]. Further plants which may be mentioned are the genus and species Argania spinosa, Arnebia griffithii, Adansonia digitata, Orbignya martiana, Carum carvi, Bertholletia excelsa, Aleurites moluccana, Hydnocarpus 65 kursii, Salvia hispanica, Vitis vinifera, Corvlus avellana, Humulus lupus, Hyptis spicigera and Shorea stenoptera.

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Plants which are advantageously used in the process according to the invention are transgenic plants such as dicotyledonous or monocotyledonous plants. Plants which are especially advantageously used in the process according to the invention are transgenic plants which belong to the oil-producing plants, that is to say which are used for the production of oils, such as, preferably, oil fruit crops 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, mullein; thistle, wild roses, hazelnut; almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perrenial grasses and fodder crops.

Preferred plants according to the invention are oilseed and oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, Indian mustard, mustard, hemp, castor-oil plant, olive, *Calendula, Punica*, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, Indian mustard, *Camelina* or hemp.

It is advantageous for the above-described processes according to the invention to additionally introduce, into the plant, further nucleic acids which encode enzymes of the fatty acid or lipid metabolism, in addition to the nucleic acids introduced in steps (a) to (e) or (a) to (c) of the process, and the optionally introduced nucleic acid sequences which encode the ω 3-desaturases and/or the Δ 12-desaturases.

In principle, all genes of the fatty acid or lipid metabolism can be used in the process for the production of polyunsaturated fatty acids, advantageously in combination with the Δ 5-elongase(s), Δ 6-elongase(s) and/or ω 3-desaturases [for the purposes of the present invention, the plural is understood as encompassing the singular and vice versa]. Genes 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), fattv acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acylcoenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously used in combination with the Δ 5-elongase, Δ 6-elongase and/or ω 3-desaturase. Genes selected from the group of the Δ 4-desaturases, Δ 5-desaturases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 9$ -desaturases, $\Delta 12$ desaturases, $\Delta 6$ -elongases or $\Delta 9$ -elongases are especially preferably used in combination with the above genes for the $\Delta 5$ -elongase, $\Delta 6$ -elongase and/or $\omega 3$ -desaturase, it being possible to use individual genes or a plurality of genes in combination. The abovementioned genes are advantageously used in combination with the $\Delta 6$ -elongase, $\Delta 5$ -elongase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase and/or $\Delta 12$ -desaturase used in accordance with the invention.

Genes selected from the group of the Δ 8-desaturases, Δ 9-desaturases, Δ 5-elongase or Δ 9-elongases are especially preferably used in combination with the abovementioned genes.

Owing to the enzymatic activity of the nucleic acids used 5 in the process according to the invention which encode polypeptides with $\Delta 6$ -elongase, $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and/or A12-desaturase activity, advantageously in combination with nucleic acid sequences which encode polypeptides of the fatty acid or lipid metabolism, such as polypeptides with $\Delta 8$ -desaturase, or $\Delta 5$ - or $\Delta 9$ -elongase activity, a wide range of polyunsaturated fatty acids can be produced in the process according to the invention. Depending on the choice of plants used for the process according to the invention, mixtures of the various polyunsaturated fatty 15 acids or individual polyunsaturated fatty acids, such as EPA or ARA, 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), fatty acids which are derived from C18:2-fatty acids, such as GLA, DGLA or ARA, or 20 fatty acids which are derived from C18:3-fatty acids, such as SDA, ETA or EPA, are thus obtained. If only linoleic acid $(=LA, C18:2^{\Delta9,12})$ is present as unsaturated fatty acid in the plant used for the process, the process can only afford GLA, DGLA and ARA as products, all of which can be present as 25 free fatty acids or in bound form. If only α -linolenic acid $(=ALA, C18:3^{\Delta9,12,15})$ is present as unsaturated fatty acid in the plant used for the process, as is the case, for example, in linseed, the process can only afford SDA, ETA or EPA as products, all of which can be present as free fatty acids or in 30 bound form, as described above.

Owing to the activity of $\Delta 6$ -desaturase and $\Delta 6$ -elongase, products formed are, for example, GLA and DGLA, or SDA and ETA, respectively, depending on the starting plant and the unsaturated fatty acid present therein. DGLA or ETA or 35 mixtures of these are preferentially formed. If $\Delta 5$ -desaturase is additionally introduced into the plant, ARA and/or EPA are also formed. If, moreover, genes which encode a Δ 5-elongase and/or Δ 4-desaturase activity are additionally introduced, the fatty acids DPA and/or DHA can be produced 40 in the process according to the invention. Advantageously, only ARA, EPA and/or DHA or mixtures of these are synthesized, depending on the fatty acid present in the plant, which acts as starting substance for the synthesis. Since biosynthetic cascades are involved, the end-products in 45 question are not present in pure form in the organisms. Small amounts of the precursor compounds are always additionally present in the end product. These small amounts amount to less than 20% by weight, advantageously less than 1.5% by weight, especially advantageously less than 10% by weight, 50 most advantageously less than 5, 4, 3, 2 or 1% by weight, based on the end products DGLA, ETA or their mixtures, or ARA, EPA or their mixtures, or ARA, EPA, DHA or their mixtures.

In addition to the production directly in the plant, of the 55 starting fatty acids for the enzymes used in the process of the invention, the fatty acids can also be fed externally. The production in the plant is preferred for reasons of economy. Substrates which are preferred for the production of ARA are linoleic acid (C18:2^{Δ 9,12}), γ -linolenic acid (C18:3^{Δ 8,9,12}) 60 and dihomo- γ -linolenic acid (20:3^{Δ 8,11,14}). Substrates which are preferred for the production of EPA are linolenic acid (C18:3^{Δ 9,12,15}), stearidonic acid (C18:4^{Δ 6,9,12,15}) and eicosatetraenoic acid (C20:4^{Δ 8,11,14,17}). Substrates which are preferred for the production of DHA are linolenic acid (C18: 3^{Δ 9,12,15}), stearidonic acid (C18:4^{Δ 6,9,12,15}), eicosatetraenoic acid (C20:4^{Δ 8,11,14,17}), EPA and DPA.

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In comparison with the human elongases or elongases from non-human animals, such as those from Oncorhyn*chus, Xenopus* or *Ciona*, the Δ 5-elongases according to the invention have the advantageous characteristic that they do not elongate C22-fatty acids to the corresponding C24-fatty acids. Furthermore, they advantageously do not convert fatty acids with a double bond in the $\Delta 6$ -position, as is the case with the human elongases or the elongases from non-human animals. Especially advantageously $\Delta 5$ -elongases preferentially only convert unsaturated C20-fatty acids. These advantageous Δ 5-elongases contain some putative transmembrane helices (5-7). Advantageously, only C₂₀-fatty acids with one double bond in the Δ 5-position are converted, with ω 3-C₂₀fatty acids being preferred (EPA). Moreover, in a preferred embodiment of the invention, they have the characteristic that, besides the $\Delta 5$ -elongase activity, they advantageously have no, or only relatively low, $\Delta 6$ -elongase activity. In contrast, the human elongases or non-human animal elongases have approximately the same activity towards fatty acids with a $\Delta 6$ - or $\Delta 5$ -double bond. These advantageous elongases are referred to what are known as monofunctional elongases. In contrast, the human elongases or the nonhuman animal elongases are referred to as multifunctional elongases, which, besides the abovementioned substrates, also convert monounsaturated $\mathrm{C}_{16}\text{-}$ and $\mathrm{C}_{18}\text{-}\text{fatty}$ acids, for example with $\Delta 9$ - or $\Delta 11$ -double bonds. In a yeast feeding text, in which EPA was added to the yeast as the substrate, the monofunctional elongases convert at least 15% by weight of the added EPA into docosapentaenoic acid (DPA, C22: $5^{\Delta7,10,13,16,19}$), advantageously at least 20% by weight, especially, advantageously at least 25% by weight. If v-linolenic acid (=GLA, C18:3^{Δ6,9,12} is added as the substrate, this acid is advantageously not elongated at all. Likewise, C18:3^{Δ 6,9,12} is not elongated. In another advantageous embodiment, less than 60% by weight of the added GLA is converted into dihomo-Y-linolenic acid (= $C20:3^{\Delta 8,11,14}$), advantageously less than 55% by weight, preferably less than 50% by weight, especially advantageously less than 45% by weight, very especially advantageously less than 40% by weight. In a further, very preferred embodiment of the Δ 5-elongase activity according to the invention, GLA is not converted.

FIGS. 27 and 28 show the measured substrate specificities of the various elongases. FIG. 27 shows the specificities of the multifunctional elongases from Xenopus laevis (FIG. 27 A), Ciona intestinalis (FIG. 27 B) and Oncorhynchus mykiss (FIG. 27 C). All these elongases convert a broad substrate spectrum. In the process according to the invention, this can lead to by-products, which must be converted by further enzymatic activities. This is why these enzymes are less preferred in the process according to the invention. The preferred monofunctional elongases and their substrate specificity are shown in FIG. 28. FIG. 28 A shows the specificity of the Ostreococcus tauri Δ 5-elongase. This enzyme only converts fatty acids with a double bond in the $\Delta 5\text{-position}.$ Advantageously, only $\mathrm{C}_{20}\text{-}\mathrm{fatty}$ acids are converted. A similarly high substrate specificity is shown by the Thallasiosira pseudonana Δ 5-elongase (FIG. 28. C). Both the Ostreococcus tauri Δ 6-elongase (FIG. 28 B) as that of Thallasiosira pseudonana (FIG. 28 D) advantageously only convert fatty acids with a double bond in the $\Delta 6$ -position. Advantageously, only C18-fatty acids are converted. The Δ 5-elongases from Arabidopsis thaliana and Euglena gracilis are also distinguished by their specificities.

Likewise, advantageous $\Delta 6$ -elongases according to the invention are distinguished by a high specificity, that is to say that C_{18} -fatty acids are preferentially elongated. They

advantageously convert fatty acids with a double bond in the Δ 6-position. Especially advantageous Δ 6-elongases advantageously convert C₁₈-fatty acids with three or four double bonds in the molecule, which fatty acids must comprise a double bond in the $\Delta 6$ -position. Moreover, in a preferred embodiment of the invention, they have the characteristic that, besides the $\Delta 6$ -elongase activity, they advantageously have no, or only relatively low, $\Delta 5$ -elongase activity. In contrast, the human elongases or non-human animal elongases have approximately the same activity towards fatty 10 acids with a $\Delta 6$ - or $\Delta 5$ -double bond. These advantageous elongases are referred to as what are known as monofunctional elongases. In contrast, the human elongases or the non-human animal elongases are referred to as multifunctional elongases, which, besides the abovementioned sub- 15 strates, also convert monounsaturated C16- and C18-fatty acids, for example with $\Delta 9$ - or $\Delta 11$ -double bonds. In a yeast feeding text, in which EPA has been added to the yeasts as the substrate, the monofunctional elongases convert at least 10% by weight of the added α -linolenic acid (=ALA, 20) C18:3^{Δ 9,12,15}) or at least 40% by weight of added γ -linolenic acid (=GLA, C18:3^{Δ 6,9,12}), advantageously at least 20% by weight and 50% by weight, respectively, especially advantageously at least 25% by weight and 60% by weight, respectively. It is especially advantageous that C18:4 $^{\Delta 6, \bar{9}, 12}$, 25 15 (stearidonic acid) is also elongated. Here, SDA is converted to at least 40% by weight, advantageously to at least 50% by weight, especially advantageously to at least 60% by weight, very especially advantageously to at least 70% by weight. Especially advantageous $\Delta 6$ -elongases show no, or 30 only very low activity (less than 0.1% by weight conversion rate) toward the following substrates: $C18:1^{\Delta6}$, $C18:1^{\Delta9}$, $C18:1^{\Delta11}$, $C20:2^{\Delta11,14}$, $C20:3^{\Delta11,14,17}$, $C20:3^{\Delta8,11,14}$, $C20:4^{\Delta5,8,11,14}$, $C20:5^{\Delta5,8,11,14,17}$ or $C22:4^{\Delta7,10,13,16}$.

FIGS. **29** and **30** and Table 21 show the measured 35 substrate specificities of the various elongases.

In comparison with the known ω 3-desaturase, the ω 3-desaturase used in the process according to the invention has the advantageous characteristic that it is capable of desaturating a broad spectrum of ω 6-fatty acids, with C₂₀- and 40 $C_{22}\mbox{-fatty}$ acids such as $C_{20:2}\mbox{-},\ C_{20:3}\mbox{-},\ C_{20:4}\mbox{-},\ C_{22:4}\mbox{-}$ or $C_{22:5}\mbox{-fatty}$ acids being preferentially desaturated. However, the shorter $\mathrm{C}_{18}\text{-}\mathrm{fatty}$ acids such as $\mathrm{C}_{18:2}\text{-}\mathrm{or}\ \mathrm{C}_{18:3}\text{-}\mathrm{fatty}$ acids are also advantageously desaturated. Owing to these characteristics of ω 3-desaturase, it is advantageously possible to 45 shift the fatty acid spectrum within an organism, advantageously within a plant or a fungus, from the ω 6-fatty acids towards the ω 3-fatty acids. The ω 3-desaturase according to the invention preferentially desaturates C₂₀-fatty acids. Within the organism, these fatty acids are converted to at 50 least 10%, 15%, 20%, 25% or 30% from the existing fatty acid pool to give the corresponding ω 3-fatty acids. In comparison with the C_{18} -fatty acids, the activity of ω 3-desaturase is lower by a factor of 10, that is to say only approximately 1.5 to 3% of the fatty acids present in the fatty 55 acid pool are converted into the corresponding ω 3-fatty acids. Preferred substrates of the w3-desaturase according to the invention are the ω 6-fatty acids bound in phospholipids. With reference to the desaturation of dihomo-y-linolenic acid $[C_{20:4}^{\Delta 8,11,14}]$, FIG. 19 shows clearly that ω 3-desatu- 60 rase advantageously does not differentiate between fatty acids bound at the sn1 or sn2 position when desaturation takes place. Both fatty acids bound at the sn1 position and fatty acids bound in the sn2 position in the phospholipids are desaturated. Another advantage is that ω 3-desaturase con-65 verts a broad range of phospholipids such as phosphatidylcholine (=PC), phosphatidylinositol (=PIS) or phosphatidy-

lethanolamine (=PE). Finally, desaturation products are also found in the neutral lipids (=NL), i.e. in the triglycerides.

In comparison with the known Δ 4-desaturases, Δ 5-desaturases and Δ 6-desaturases, the advantage of the Δ 4-desaturases, Δ 5-desaturases and Δ 6-desaturases used in the process according to the invention is that they can convert fatty acids which are bound to phospholipids or CoA-fatty acid esters, advantageously CoA-fatty acid esters.

The $\Delta 12$ -desaturases used in the process according to the invention advantageously convert oleic acid (C18:1^{$\Delta 9$}) into linoleic acid (C18:2^{$\Delta 9$,12}) or C18:2^{$\Delta 6,9$} into C18:3^{$\Delta 6,9,12$} (=GLA). The $\Delta 12$ -desaturases used advantageously convert fatty acids which are bound to phospholipids or CoA-fatty acid esters, advantageously those which are bound to CoA-fatty acid esters.

Owing to the enzymatic activity of the nucleic acids used in the process according to the invention which encode polypeptides with Δ 5-elongase, Δ 6-elongase and/or ω 3-desaturase activity, advantageously in combination with nucleic acid sequences which encode polypeptides of the fatty acid or lipid metabolism, such as additionally polypeptides with $\Delta 4$ -, $\Delta 5$ -, $\Delta 6$ -, $\Delta 8$ -, $\Delta 12$ -desaturase or $\Delta 5$ -, $\Delta 6$ or Δ 9-elongase activity, a very wide range of polyunsaturated fatty acids can be produced in the process according to the invention. Depending on the choice of the advantageous plants used for the process according to the invention, mixtures of the various polyunsaturated fatty acids or individual polyunsaturated fatty acids such as EPA, ARA 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), fatty acids which are derived from C18:2-fatty acids, such as GLA, DGLA or ARA, or which are derived from C18:3-fatty acids, such as SDA, ETA, EPA or DHA, are thus obtained. If only linoleic acid (=LA, C18: $2^{\Delta 9,12}$) is present as unsaturated fatty acid in the plant used for the process, the process can only afford GLA, DGLA and ARA as products, all of which can be present as free fatty acids or in bound form. By expressing the additional w3-desaturase in plants, the fatty acid spectrum can be shifted towards α -linolenic acid, DPA and DHA. However, this shift in the fatty acid spectrum is only relatively limited. More advantageous is such a shift in plants which, as described hereinbelow, already have a high α -linolenic acid content. If only α -linolenic acid (=ALA, C18:3^{Δ 9,12,15}) is present as unsaturated fatty acid in the plant, as is the case, for example, in linseed, the process can only afford SDA, ETA. EPA and/or DHA, which, as described above, may be present as free fatty acids or in bound form. Owing to the modification of the activity of the enzyme $\Delta 5$ -elongase which plays a role in the synthesis, advantageously in combination with $\Delta 4$ -, $\Delta 5$ -, $\Delta 6$ -, $\Delta 12$ -desaturase and/or $\Delta 6$ -elongase, or $\Delta 4$ -, $\Delta 5$ -, $\Delta 8$ -, $\Delta 12$ -desaturase, and/or $\Delta 9$ -elongase, it is possible to produce; in a targeted fashion, only individual products in the abovementioned plants. Owing to the activity of $\Delta 6$ -desaturase and $\Delta 6$ -elongase, for example, GLA and DGLA, or SDA and ETA, are formed, depending on the starting plant and unsaturated fatty acids. DGLA or ETA or mixtures of these are preferentially formed. If Δ 5-desaturase, Δ 5-elongase and Δ 4-desaturase are additionally introduced into the organisms, advantageously into the plant, ARA, EPA and/or DHA are additionally formed. This also applies to organisms into which $\Delta 8$ -desaturase and $\Delta 9$ -elongase have previously been introduced. Advantageously, only ARA, EPA or DHA or their mixtures are synthesized, depending on the fatty acid present in the plant, which acts as starting substance for the synthesis. Since biosynthetic cascades are involved, the end products in question are not present in pure form in the organisms. Small amounts of the precursor compounds are always additionally present in the end product. These small amounts amount to less than 20% by weight, advantageously less than 15% by weight, especially advantageously less than 10% by weight, very especially advantageously less than 5, 4, 3, 2, or 1% by weight, based on the end product DGLA, ETA or their mixtures, or ARA, EPA, DHA or their mixtures, advantageously EPA or DHA or their mixtures.

The nucleic acid with the SEQ ID NO: 53, which is derived from trout and which can be used in the process according to the invention, encodes a protein with high specificity for the two C18:4^{Δ 6,9,12,15}</sub> and C20:5^{Δ 5,8,11,14,17}-fatty acids, which are precursors for the 15 synthesis of DHA (precursors and synthesis of DHA, see FIG. 1). However, other fatty acids too are elongated by the enzyme. The protein encoded by SEQ ID NO: 53 thus has specificity for Δ 6- and Δ 5-fatty acids with additionally one ω 3-double bond (FIG. 2). Δ 5-elongase has a keto-acyl-CoA 20 synthase activity which advantageously elongates fatty acid residues of acyl-CoA esters by 2 carbon atoms.

The synthesis of DHA in yeast (*Saccharomyces cerevi*siae) was detected by the gene product of the abovementioned fish Δ 5-elongase gene and further Δ 5-elongases, the 25 Δ 5-desaturase from *Phaeodactylum* and the Δ 4-desaturase from *Euglena* (FIG. **3**).

In addition to the production directly in the transgenic organism, advantageously in the transgenic plant, of the starting fatty acids for the Δ 5-elongases, Δ 6-elongases, 30 Δ 9-elongases, Δ 4-desaturases, Δ 5-desaturases, Δ 6-desaturases, $\Delta 12$ -desaturases and/or $\omega 3$ -desaturases advantageously used in the process according to the invention, the fatty acids can also be shed externally. The production in the organism is preferred for reasons of economy. Preferred 35 substrates of ω 3-desaturase are linoleic acid (C18:2^{Δ 9,12}), y-linolenic acid (C18:3^{Δ 8,9,12}), eicosadienoic acid (C20:2^{Δ 11,14}); dihomo- γ -linolenic acid (C20:3^{Δ 8,11,14}), arachidonic acid; (C20: $4^{\Delta 5,8,11,14}$), docosatetraenoic acid (C22:4^{Δ7,10,13,16}) and docosapentaenoic acid 40 $(C22:5^{\Delta4,7,10,13,15}).$

To increase the yield in the above-described process for the production of oils and/or triglycerides with an advantageously elevated content of polyunsaturated fatty acids, it is advantageous to increase the amount of starting product for 45 the synthesis of fatty acids; this can be achieved for example by introducing, into the organism, a nucleic acid which encodes a polypeptide with $\Delta 12$ -desaturase activity. This is particularly advantageous in oil-producing organisms such as those from the family of the Brassicaceae, such as the 50 genus Brassica, for example oilseed rape; the family of the Elaeagnaceae, 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 55 these organisms are only low in linoleic acid (Mikoklajczak et al., Journal of the American Oil Chemical Society, 38, 1961, 678-681), the use of the abovementioned Δ 12-desaturases for producing the starting material linoleic acid is advantageous.

Nucleic acids used in the process according to the invention are advantageously derived from plants such as algae, for example algae of the family of the Prasinophyceae such as the genera *Heteromastix*, *Mammella*, *Mantoniella*, *Micromonas*, *Nephroselmis*, *Ostreococcus*, *Prasinocladus*, 65 *Prasinococcus*, *Pseudoscourfielda*, *Pycnococcus*, *Pyramimonas*, *Scherffelia* or *Tetraselmis* such as the genera and

species Heteromastix longifillis, Mamiella gilva, Mantoniella squamata, Micromonas pusilla, Nephroselmis olivacea, Nephroselmis pyriformis, Nephroselmis rotunda, Ostreococcus tauri, Ostreococcus sp. Prasinocladus ascus, Prasinocladus lubricus, Pycnococcus provasolii, Pyramimonas amylifera, Pyramimonas disomata, Pyramimonas obovata, Pyramimonas orientalis, Pyramimonas parkeae, Pyramimonas spinifera, Pyramimonas sp., Tetraselmis apiculata, Tetraselmis carteriaformis, Tetraselmis chui, Tetraselmis convolutae, Tetraselmis desikacharyl, 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 from algae of 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 geniculate, Euglena gracilis, Euglena mixocylindrica, Euglena rostrifera Euglena viridis, Colacium stentorium, Trachelomonas cylindrica or Trachelomonas volvocina. The nucleic acid sequences used in the process can also advantageously be derived from algae, such as the alga Porphyridium cruentum, Isochrysis galbana or Chlorella minutissima, Chlorella vulgaris, Thraustochytrium aureum or Nannochloropsis oculata. The nucleic acids used are advantageously derived from algae of the genera Euglena, Mantoniella or Ostreococcus.

Further advantageous plants as sources for the nucleic acid sequences used in the process according to the invention 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 stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Thraustochytrium, Phytophthora, Eritomophthora, Mucor or Mortierella, bacteria such as Shewanella, yeasts or animals such as nematodes such as Caenorhabditis, insects, frogs, sea cucumber or fish. The isolated nucleic acid sequences according to the invention are advantageously derived from an animal of the order of the vertebrates. Preferably, the nucleic acid sequences are derived from the classes of the Vertebrata; Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus or Vertebrata, Amphibia, Anura, Pipidae, Xenopus or Evertebrata such as Protochordata, Tunicata, Holothuroidea, Cionidae such as Amaroucium constellatum, Botryllus schlosseri, Ciona intestinalis, Molgula citrina, Molgula manhattensis, Perophora viridis or Stvela partita. The nucleic acids are especially advantageously derived from fungi, animals, or from plants such as algae or mosses, preferably from the order of the Salmoniformes, such as the family of the Salmonidae, such as 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 60 Phaeodactylum or from algae such as Crypthecodinium.

Advantageous nucleic acid used in the process according to the invention can also be derived from microorganisms such as fungi such as the genus *Mortierella*, *Phytium*, for example the genus and species *Mortierella alpiina*, *Mortierella elongata*, *Phytium irregulare*, *Phytium ultimum* or bacteria such as the genus *Shewanella*, for example the genus and species *Shewanella hanedai*. The process according to the invention advantageously employs the abovementioned nucleic acid sequences or their derivatives or homologs which encode polypeptides which retain the enzymatic activity of the proteins encoded by nucleic acid sequences. These sequences, individually or in 5 combination with the nucleic acid sequences which encode $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase and/or ω -3-desaturase, are cloned into expression constructs and used for the introduction into, and expression constructs make possible an advantageous optimal synthesis of the polyunsaturated fatty acids produced in the process according to the invention.

In a preferred embodiment, the process furthermore comprises the step of obtaining a transgenic plant which com-15 prises the nucleic acid sequences used in the process, where the plant is transformed with a nucleic acid sequence according to the invention which encodes the Δ 12-desaturase, Δ 4-desaturase, Δ 5-desaturase, Δ 6-desaturase, Δ 5-elongase, Δ 6-elongase and/or ω 3-desaturase, a gene construct or a 20 vector as described below, alone or in combination with further nucleic acid sequences which encode proteins of the fatty acid or lipid metabolism. In a further preferred embodiment, this process furthermore comprises the step of obtaining the oils, lipids or free fatty acids from the seed of the 25 plant, such as, for example, the seed of an oil crop, such as, for example, peanut, oilseed rap, canola, linseed, hemp, peanut, soybean, safflower, hemp, sunflowers or borage.

In the case of plant cells, plant tissue or plant organs, "growing" is understood as meaning, for example, the 30 cultivation on or in a nutrient medium, or of the intact plant on or in a substrate, for example in a hydroponic culture, potting compost or on arable land.

The invention furthermore relates to gene constructs which comprise the nucleic acid sequences according to the 35 invention which encode a $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, Δ 5-elongase or Δ 6-elongase, the nucleic acid being linked functionally with one or more regulatory signals. In addition, the gene construct may comprise further biosynthesis genes of the fatty acid or lipid metabolism selected from the 40 group consisting of acyl-CoA dehydrogenase(s), acyl-ACP protein acyl-ACP carrier desaturase(s), [=acv1 thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), 45 acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s). Biosynthesis genes of the fatty acid or lipid metabolism selected from the group $\Delta 8$ -desaturase, $\Delta 9$ -de- 50 saturase, $\Delta 9$ -elongase or $\omega 3$ -desaturase are advantageously additionally present.

The nucleic acid sequences used in the process which encode proteins with Δ 5-desaturase, Δ 6-desaturase, Δ 12desaturase, Δ 5-elongase or Δ 6-elongase activity are advan-55 tageously introduced into the plant alone or, preferably, in combination with an expression cassette (=nucleic acid construct) which makes possible the expression of the nucleic acids in the plant. The nucleic acid construct can comprise more than one nucleic acid sequence with an 60 enzymatic activity, for example, of a Δ 12-desaturase, Δ 5-desaturase, Δ 6-desaturase, Δ 5-elongase and/or Δ 6-elongase.

To introduce the nucleic acids into the gene constructs, the nucleic acids used in the process are advantageously amplified and ligated in the known manner. Preferably, a proce-65 dure following the protocol for Pfu DNA polymerase or a Pfu/Taq DNA polymerase mixture is followed. The primers

are selected taking into consideration the sequence to be amplified. The primers should expediently be chosen in such a way that the amplificate comprises the entire codogenic sequence from the start codon to the stop codon. After the amplification, the amplificate is expediently analyzed. For example, a gel-electrophoretic separation can be carried out, which is followed by a quantitative and a qualitative analysis. Thereafter, the amplificate can be purified following a standard protocol (for example Qiagen). An aliquot of the purified amplificate is then available for the subsequent cloning step.

Suitable cloning vectors are generally known to the skilled worker. These include, in particular, vectors which are capable of replication in microbial systems, that is to say mainly vectors which ensure efficient cloning in yeasts or fungi and which make possible the stable transformation of plants. Those which must be mentioned in particular are various binary and cointegrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they comprise at least the vir genes required for the Agrobacteriummediated transformation and the T-DNA-delimiting sequences (T-DNA border). These vector systems preferably also comprise further cis-regulatory regions such as promoters and terminator sequences 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 coli and in Agrobacterium. These binary vectors include vectors from the series pBIB-HYG, pPZP, pBecks, pGreen. In accordance with the invention, Bin19, pBI101, pBinAR, pGPTV and pCAMBIA are used by preference. An overview of the binary vectors and their use is found in Hellens et al, Trends in Plant Science (2000) 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 ligated 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 functionally with regulatory sequences. The regulatory sequences include, in particular, plant sequences such as promoters and terminator sequences. The constructs can advantageously be stably propagated in microorganisms, in particular in E. coli and Agrobacterium tumefaciens, under selection conditions and make possible a transfer of heterologous DNA into plants or microorganisms.

The nucleic acids used in the process can be introduced into plants, advantageously using cloning vectors, and thus be used in the transformation of plants such as those which are published and cited therein: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Fla.), 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, Eds.: 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, Eds.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225. Thus, the nucleic acids 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 5 and/or more efficient PUFA producers.

A series of mechanisms by which a modification of the Δ 12-desaturase, Δ 5-elongase, Δ 6-elongase, Δ 5-desaturase and/or $\Delta 6$ -desaturase protein is possible exists, so that the yield, production and/or production efficiency of the poly-10 unsaturated fatty acids in a plant, preferably in an oilseed plant or oil crop, can be influenced directly owing to this modified protein. The number or activity of the $\Delta 12$ -Desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase or Δ 5-desaturase proteins or genes can be increased, so that 15 greater amounts of the gene products and, ultimately, greater amounts of the compounds of the general formula I are produced. A de novo synthesis in a plant which has lacked the activity and ability to biosynthesize the compounds prior to introduction of the corresponding gene(s) is also possible. 20 This applies analogously to the combination with further desaturases or elongases or further enzymes of the fatty acid and lipid metabolism. The use of various divergent sequences, i.e. sequences which differ at the DNA sequence level, may also be advantageous in this context, or else the 25 use of promoters which make possible a different gene expression in the course of time, for example as a function of the degree of maturity of a seed or an oil-storing tissue.

Owing to the introduction of a combination of $\Delta 12$ desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase and/or 30 $\Delta 5$ -desaturase genes into the 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 composition. Likewise, the number or activity of other genes which 35 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 40 ability of the cells to produce PUFAs is enhanced further. By optimizing the activity or increasing the number of one or more $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase or $\Delta 5$ -desaturase genes which are involved in the biosynthesis of these compounds, or by destroying the 45 activity of one or more genes which are involved in the degradation of these compounds, an enhanced vield, production and/or production efficiency of fatty acid and lipid molecules in plants is made possible.

The nucleic acid sequences used in the process are 50 advantageously introduced into an expression cassette which makes possible the expression of the nucleic acids in plants.

In doing so, the nucleic acid sequences which encode $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase or 55 $\Delta 5$ -desaturase are linked functionally with one or more regulatory signals, advantageously for enhancing gene expression. These regulatory sequences are intended to make possible the specific expression of the genes and proteins. Depending on the host organism, this may mean, 60 for example, that the gene is expressed and/or overexpressed only after induction has taken place, or else that it is expressed and/or overexpressed immediately. For example, these regulatory sequences take the form of sequences to which inductors or repressors bind, thus controlling the 65 expression of the nucleic acid. In addition to these novel regulatory sequences, or instead of these sequences, the

natural regulatory elements of these sequences may still be present before the actual structural genes and, if appropriate, may have been genetically modified in such a way that their natural regulation is eliminated and the expression of the genes is enhanced. These, modified promoters can also be positioned on their own before the natural gene in the form of part-sequences (=promotor with parts of the nucleic acid sequences used in accordance with the invention) in order to enhance the activity. Moreover, the gene construct may advantageously also comprise one or more what are known as enhancer sequences in operable linkage with the promoter, which make possible an enhanced expression of the nucleic acid sequence. Additional advantageous sequences, such as further regulatory elements or terminator sequences, may also be inserted at the 3' end of the DNA sequences.

The $\Delta 12$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 6$ -elongase genes 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 expressed together in the host plant. 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 host genome when the genes to be expressed are present together in one gene construct.

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.

In a further embodiment of the invention, one or more gene constructs comprising one or more sequences which are defined by SEQ ID NO: 11, SEQ ID NO: 27, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 199, SEQ ID NO: 201 or their derivatives and which encode polypeptides as shown in SEQ ID NO: 12, SEQ ID NO: 28, SEQ ID NO: 194, SEQ ID NO: 196, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202 are present. The abovementioned $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase or $\Delta 5$ -desaturase proteins advantageously lead to a desaturation or elongation of fatty acids, the substrate advantageously having one, two, three or four double bonds and advantageously 18, 20 or 22 carbon atoms in the fatty acid molecule. The same applies to their homologs, derivatives or analogs which are linked functionally with one or more regulatory signals, preferably for enhancing gene expression.

In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. It is also possible and advantageous to use synthetic promoters, either in addition or alone, in particular when they mediate seed-specific expression, such as those described in WO 99/16890.

In order to achieve a particularly high PUFA content, especially in transgenic plants, the PUFA biosynthesis genes should advantageously be expressed in oilseeds in a seedspecific manner. To this end, seed-specific promoters can be used, or those promoters which are active in the embryo and/or in the endosperm. In principle, seed-specific promoters can be isolated both from dicotyledonous and from monocotyledanous plants. Preferred promoters are listed hereinbelow: USP (=unknown seed protein) and vicilin (Vicia faba) [Bäumlein et al., Mol. Gen. Genet., 1991, 225(3)], napin (oilseed rape) [U.S. Pat. No. 5,608,152], conlinin (linseed) [WO 02/102970], acyl carrier protein (oilseed rape) [U.S. Pat. No. 5,315,001 and WO 92/18634], 5 oleosin (Arabidopsis thaliana) [WO 98/45461 and WO 93/20216], phaseolin (Phaseolus vulgaris) [U.S. Pat. No. 5,504,200], Bce4 [WO 91/13980], Iegumes B4 (LegB4 promoter) [Bäumlein et al., Plant J., 2,2, 1992], Lpt2 and Ipt1 (barley) [WO 95/15389 and WO95/23230], seed-spe- 10 cific promoters from rice, maize and wheat [WO 99/16890], Amy32b, Amy 6-6 and aleurain [U.S. Pat. No. 5,677,474], Bce4 (oilseed rape) [U.S. Pat. No. 5,530,149], glycinin (soybean) [EP 571 741], phosphoenol pyruvate carboxylase (soybean) [JP 06/62870], ADR12-2 (soybean) [WO 15 98/08962], isocitrate lyase (oilseed rape) [U.S. Pat. No. 5,689,040] or α -amylase (barley) [EP 781 849].

Plant gene expression can also be facilitated via a chemically inducible promoter (see a review in Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically 20 inducible promoters are particularly suitable when it is desired that gene expression should take place in a timespecific manner. Examples of such promoters are a salicylicacid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and 25 an ethanol-inducible promoter.

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 $\Delta 12$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase and/or $\Delta 5$ -desaturase 30 and which are used in the process should be expressed under the control of a separate promoter, preferably a promoter which differs from the other promoters, since repeating sequence motifs can lead to instability of the T-DNA, or to recombination events. In this context, the expression cas- 35 sette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site, advantageously in a polylinker, for insertion of the nucleic acid to be expressed, and, if appropriate, a terminator sequence, is positioned behind the polylinker. This sequence is repeated 40 several times, preferably three, four, five, six or seven times, so that up to seven genes can be combined in one construct and introduced into the transgenic plant in order to be expressed. Advantageously, the sequence is repeated up to four times. To express the nucleic acid sequences, the latter 45 are inserted behind the promoter via a suitable cleavage site, for example in the polylinker. Advantageously, each nucleic acid sequence has its own promoter and, if appropriate, its own terminator sequence. Such advantageous constructs are disclosed, for example, in DE 101 02 337 or DE 101 02 338. 50 However, it is also possible to insert a plurality of nucleic acid sequences behind a shared promoter and, if appropriate, before a shared terminator sequence. 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 55 nucleic acid sequence can be inserted at the first or last position in the cassette without its expression being substantially influenced thereby. Advantageously, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminator sequences can be used in 60 a nucleic acid molecule which is capable of transporting the expression cassette. However, it is also possible to use only one type of promoter in the cassette, which, however, may lead to undesired recombination events.

As described above, the transcription of the genes which have been introduced should advantageously be terminated 65 by suitable terminator sequences 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 OCS1 terminator sequence. As is the case with the promoters, different terminator sequences should be used for each gene.

As described above, the gene construct can also comprise further genes to be introduced into the plants. It is possible and advantageous to introduce into the host plants, and to express, 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; however, these genes can also be present on one or more further nucleic acid constructs. A biosynthesis gene of the fatty acid or lipid metabolism which is preferably chosen is a gene from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [=acv1 carrier protein desaturase(s). acvl-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, ω 3-desaturase, Δ 8-desaturase, Δ 4-desaturase, Δ 9-desaturase, $\Delta 5$ -elongase 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.

Here, the regulatory sequences or factors can, as described above, preferably have a positive effect on, and thus enhance, the gene expression of the genes which have been introduced. Thus, enhancement of the regulatory elements can advantageously take place at the transcriptional level by using strong transcription signals such as promoters and/or enhancers. However, an enhanced translation is also possible, for example by improving the stability of the mRNA. In principle, the expression cassettes can be used directly for introduction into the plants or else be introduced into a vector.

These advantageous vectors, preferably expression vectors, comprise the nucleic acids which encode the $\Delta 12$ desaturases, $\Delta 6$ -desaturases, $\Delta 5$ -elongases, $\Delta 6$ -elongases or $\Delta 5$ -desaturases and which are used in the process, or else a nucleic acid construct which comprises the nucleic acid used either alone or in combination with further biosynthesis genes of the fatty acid or lipid metabolism such as the acyl-CoA:lysophospholipid acyltransferases, w3-desaturases, $\Delta 8$ -desaturases, $\Delta 9$ -desaturases, $\omega 3$ -desaturases, Δ 4-desaturases, Δ 5-elongases and/or Δ 9-elongases.

As used in the present context, the term "vector" refers to 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 10 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 15 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 acids or the described 20 gene construct used in accordance with the invention 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, selected on the basis of the host cells used for the expression, which 25 regulatory sequence(s) is/are linked functionally with the nucleic acid sequence to be expressed. In a recombinant expression vector, "linked functionally" or "in operable linkage" means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the 30 expression of the nucleotide sequence is 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 35 achieved via a chemically inducible promoter (see a review 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 40 Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Fla., Eds.: Glick and Thompson, Chapter 7, 89-108, including the references cited 45 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 50 design of the expression vector can depend on factors such as the choice of host cell to be transformed, the desired expression level of the protein and the like.

In a further embodiment of the process, the $\Delta 12$ -desaturases, $\Delta 6$ -desaturases, $\Delta 5$ -elongases, $\Delta 6$ -elongases and/or 55 $\Delta 5$ -desaturases can 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 60 comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1195-1197; and Bevan, M. W. (1984) "Binary Agrobacterium vectors for plant 65 transformation", Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol.

1, Engineering and Utilization, Eds.: 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 which are linked functionally so that each sequence can fulfill 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., EMBO J. 3 (1984) 835 et seq.), which is known as octopine synthase, or functional equivalents thereof, but all other terminator sequences 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 functionally, 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 gene to be expressed must be linked functionally with a suitable promoter which triggers gene expression with the correct planning or in a cell- or tissue-specific manner. Utilizable promoters are constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (see also U.S. Pat. No. 5,352,605 and WO 84/02913), or constitutive plant promoters, such as the promoter of the Rubisco small subunit, which is described in U.S. Pat. No. 4,962,028.

As described above, plant gene expression can also be 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 (U.S. Pat. No. 5,187,267), the chill-inducible potato alphaamylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Especially preferred are those promoters which bring about the gene expression in tissues and organs in which the biosynthesis of fatty acids, lipids and oils takes place, in seed cells, such as cells of the endosperm and of the developing embryo. Suitable promoters are the oilseed rape napin promoter (U.S. Pat. No. 5,608,152), the linseed Conlinin promoter (WO 02/102970), the Vicia faba USP promoter (Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67), the Arabidopsis oleosin promoter (WO 98/45461), the Phaseolus vulgaris phaseolin promoter (U.S. Pat. No. 5,504, 200), the Brassica Bce4 promoter (WO 91/13980) or the legume B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable noteworthy promoters are the barley Ipt2 or Ipt1 gene promoter (WO 95/15389 and WO 95/23230) or the promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamine gene, the wheat gliadine gene, the wheat glutelin gene, the maize zeine gene, the oat glutelin gene, the sorghum kasirin gene or the rye secalin gene, which are described in WO 99/16890.

Other promoters which are also particularly suitable are ⁵ 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 are the viral RNA polymerase promoter, described in WO 95/16783 and WO 97/06250, ¹⁰ and the *Arabidopsis* clpP promoter, described in WO 99/46394.

In particular, it may be desired to bring about the multiparallel expression of the $\Delta 12$ -desaturases, $\Delta 6$ -desaturases, $\Delta 5$ -elongases, $\Delta 6$ -elongases and/or $\Delta 5$ -desaturases used in the process. Such expression cassettes can be introduced via the simultaneous transformation of a plurality of individual expression constructs or, preferably, by combining a plurality of expression cassettes on one construct. Also, a plurality 20 of vectors can be transformed with in each case a plurality of expression cassettes and then transferred into the host cell.

Other preferred sequences for the use in operable linkage in plant gene expression cassettes are targeting sequences 25 which are required for targeting the gene product into its corresponding cell compartment, for example into the vacuole, the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, elaioplasts, peroxi-30 somes and other compartments of plant cells (see a review in Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423 and references cited therein).

The process according to the invention employs the nucleic acid sequences with the SEQ ID NO: 11, SEQ ID 35 NO: 27, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 199, SEQ ID NO: 201 or their derivatives or homologs which encode polypeptides which retain the enzymatic activity of the proteins encoded by nucleic acid sequences. These sequences, individually or in combination 40 with the nucleic acid sequences which encode the other enzymes used, are cloned into expression constructs and used for the transformation into, and expression in, plants. Owing to their construction, these expression constructs make possible an advantageous optimal synthesis of the 45 polyunsaturated fatty acids produced in the process according to the invention.

In a preferred embodiment, the process furthermore comprises the step of obtaining a cell or an intact plant which comprises the nucleic acid sequences used in the process, 50 where the cell and/or the plant is transformed with a nucleic acid sequence encoding a polypeptide with a $\Delta 12$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 6$ -elongase activity, a gene construct or a vector as described above, alone or in combination with further 55 nucleic acid sequences which encode proteins of the fatty acid or lipid metabolism. The resulting cell is advantageously a cell of an oil-producing organism such as an oil crop, such as, for example, peanut, oilseed rape, canola, linseed, hemp, peanut, soybean, safflower, hemp, mustard, 60 sunflowers or borage.

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 sequence according to the 65 invention or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the

invention, all those constructions brought about by recombinant methods in which either

a) the nucleic acid sequence according to the invention, or b) a genetic control sequence which is operably linked with

the nucleic acid sequence according to the invention, 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 take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning 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, most preferably at least 5000 bp. A naturally occurring expression cassette—for example the naturally occurring combination of the natural promoter of the nucleic acid sequences used in the process according to the invention with the corresponding $\Delta 12$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, U)-3-desaturase, Δ 9-elongase, Δ 6-elongase and/or Δ 5-elongase genes—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 U.S. Pat. No. 5,565,350 or WO 00/15815.

Transgenic plants for the purposes of the invention is therefore understood as meaning that the nucleic acids used in the process are not at their natural locus in the genome of the plant, it being possible for the nucleic acids 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, however, the sequence having 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 according to the invention or the nucleic acid sequences 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 acids takes place. Preferred transgenic plants are oilseed or oil fruit crops.

Plants which are suitable for use in the process according to the invention are, in principle, advantageously all plants which are capable of synthesizing fatty acids, specifically unsaturated fatty acids such as ARA, EPA and/or DHA, and which are suitable for the expression of recombinant genes. Examples are plants such as Arabidopsis, Asteraceae such as Calendula or crop plants such as soybean, peanut, castor-oil plant, sunflower, maize, cotton, flax, oilseed rape, coconut, oil palm, safflower (Carthamus tinctorius) or cacao bean. Plants which are naturally capable of synthesizing large amounts of oils are preferred, such as soybean, oilseed rape, Camelina, Indian mustard, coconut, oil palm, safflower (Carthamus tinctorius), flax, hemp, castor-oil plant, Calendula, peanut, cacao bean or sunflower or yeast such as Saccharomyces cerevisiae, with soybean, flax, oilseed rape, safflower, sunflower, Camelina, indian mustard or Calendula being especially preferred.

Further host cells which can be used for cloning the nucleic acid sequences used in the process according to the

invention are detailed in: Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990).

Expression strains which can be used, for example those with a lower protease activity, are described in: Gottesman, 5 S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 119-128.

These include plant cells and certain tissues, organs and parts of plants in all their phenotypic forms such as anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, 10 harvested material, plant tissue, reproductive tissue and cell cultures which is derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant.

Transgenic plants or advantageously the seeds thereof which comprise the polyunsaturated fatty acids in particular 15 ARA, EPA and/or DHA, 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. Plants for the process according to the invention are as meaning intact plants and all plant 20 parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about 25 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.

In principle, the process according to the invention is also suitable for the production of polyunsaturated fatty acids, in 30 particular ARA, EPA and/or DHA, in plant cell cultures, followed by obtaining the fatty acids from the cultures. In particular, they may take the form of suspension or callus cultures.

However, the compound produced in the process according to the invention can also be isolated from the plants, advantageously the plant seeds, in the form of their oils, fat, lipids and/or free fatty acids. Polyunsaturated fatty acids produced by this process, in particular ARA, EPA and/or DHA, can be harvested by harvesting the plants or plant 40 seeds either from the culture in which they grow, or from the field.

In a further preferred embodiment, this process furthermore comprises the step of obtaining the oils, lipids or free fatty acids from the plant or from the crop. The crop may, for 45 example, take the form of a greenhouse- or field-grown plant crop.

The oils, lipids or free fatty acids can be isolated via pressing or extraction of the plant parts, preferably the plant seeds. In this context, the oils, fats, lipids and/or free fatty 50 acids can be obtained by what is known as cold-beating or cold-pressing without applying heat. To allow for greater ease of disruption of the plant parts, specifically the seeds, they are previously comminuted, steamed or roasted. The seeds which have been pretreated in this manner can sub-55 sequently be pressed or extracted with solvents such as warm hexane. The solvent is subsequently removed.

Thereafter, the resulting products which comprise the polyunsaturated fatty acids are processed further, i.e. refined. In this process, substances such as the plant muci- 60 lages and suspended matter are first removed. What is known as desliming can be effected enzymatically or, for example, chemico-physically by addition of acid such as phosphoric acid. Thereafter, the free fatty acids are removed by treatment with a base, for example sodium, hydroxide 65 solution. The resulting product is washed thoroughly with water to remove the alkali remaining in the product and then

dried. To remove the pigment remaining in the product, the products are subjected to bleaching, for example using fuller's earth or active charcoal. At the end, the product is deodorized, for example using steam.

The PUFAs or LCPUFAs produced by this process are preferably C_{18} -, C_{20} - or C_{22} -fatty acid molecules, advantageously C_{20} - or C_{22} -fatty acid molecules, with at least two double bonds in the fatty acid molecule, preferably with three, four, five or six double bonds, especially preferably with four, five or six double bonds. These C_{18} -, C_{20} - or C_{22} -fatty acid molecules can be isolated from the plant in the form of an oil, a lipid or a free fatty acid. Examples of suitable plants are those mentioned above. Suitable organisms are transgenic plants.

One embodiment of the invention are therefore oils, lipids or fatty acids or fractions thereof which have been prepared by the above-described process, especially preferably oils, lipids or a fatty acid composition which comprise PUFAs and originate from transgenic plants.

The fatty acids obtained in the process are also suitable as starting material for the chemical synthesis of products of value. For example, they can be used together or alone for the production of pharmaceuticals, foodstuffs, feedstuffs or cosmetics.

As described above, these oils, lipids or fatty acids advantageously comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Advantageous polyunsaturated fatty acids which are present in the fatty acid esters or fatty acid mixtures are preferably at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% of arachidonic acid, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention advantageously comprise fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienoic acid), vernolic acid (9, 10 epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10heptadecen-8-vnoic acid), crepenvninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13coctadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (allcis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The abovementioned fatty acids are, as a rule, advantageously only found in traces in the fatty acid esters or fatty acid mixtures produced by the process according to the invention, that is to say that, based on the total fatty acids, they occur to less than 30%, preferably to less than 25%, 24%, 23%, 22% or 21%, especially preferably to less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very especially preferably to less than 4%, 3%, 2% or 1%. In a further preferred form of the invention, these abovementioned fatty

acids occur in amounts of less than 0.9%, 0.8%, 0.7%, 0.6% or 0.5%, especially preferably less than 0.4%, 0.3%, 0.2%, 0.1%, based on the total fatty acids. The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1%, based on 5 the total fatty acids, and/or no butyric acid, no cholesterol, no clupanodonic acid (=docosapentaenoic acid, C22:5^{A4,8,12,15,21}) and no nisinic acid (tetracosahexaenoic acid, C23:6^{A3,8,12,15,18,21}).

As a rule, the abovementioned fatty acids are advanta- 10 geously only found in traces in the fatty acid esters or fatty acid mixtures produced by the process according to the invention, that is to say that, based on the total fatty acids, they are found in amounts of less than 30%, preferably less than 25%, 24%, 23%, 22% or 21%, especially preferably less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very especially preferably less than 4%, 3%, 2% or 1%. In a further preferred embodiment of the invention, these abovementioned fatty acids are found relative to the total fatty acids in amounts of less than 0.9%, 0.8%, 0.7%, 0.6% or 20 0.5%, especially preferably less than 0.4%, 0.3%, 0.2%, 0.1%. 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 and/or no butyric acid, no cholesterol, no clupanodonic acid (=do- 25 cosapentaenoic acid, C22:5^{Δ 4,8,12,15,21}) and no nisinic acid (tetracosahexaenoic acid, C23: $6^{\Delta 3,8,12,15,18,21}$).

The oils, lipids or fatty acids according to the invention advantageously comprise at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%, advantageously at least 11%, 12%, 13%, 14%, 15%, 16% or 17%, especially advantageously at least 18%, 19%, 20%; 21%, 22%, 23%, 24% or 25% of ARA or at least 0.5%; 1%, 2%, 3%, 4%, 5% or 6%, advantageously at least 7%, 8%, 9%, 10% or 11%, especially advantageously at least 12%, 13%, 14%, 15%, 16%, 35 17%, 18%, 19% or 20% of EPA or at least 0.01%, 0.02%, 0.03%, 0.04% or 0.05% or 0.06%, advantageously at least 0.07%, 0.08%, 0.09% or 0.1%, especially advantageously at least 0.2%, 0.3% or 0.4% of DHA, based on the total fatty acid content of the production organism, advantageously of 40 a plant, especially advantageously of an oil crop such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean, sunflower or the abovementioned other monocotyledonous or dicotyledonous oil crops. All percentages are by weight.

Owing to the nucleic acid sequences according to the invention, or the nucleic acid sequences used in the process according to the invention, it is possible to obtain an increase in the yield of polyunsaturated fatty acids, mainly ARA and EPA, but also DHA, of at least 50, 80 or 100%, advantageously at least 150, 200 or 250%, especially advantageously at least 300, 400, 500, 600, 700, 800 or 900%, very advantageously at least 1000, 1100, 1200, 1300, 1400 or 1500% in comparison with the non-transgenic starting plant, for example a plant such as *Brassica juncea, Brassica 55 napus, Camelina sativa, Arabidopsis thanliana* or *Linum usitatissimum* when using a GC analysis for comparison purposes, see Examples.

The lipids and/or oils produced in the process according to the invention have a higher content of the unsaturated 60 fatty acids oleic acid, linoleic acid and α -linolenic acid in the sn2-position in comparison with the other positions sn1 and sn3. A higher content is understood as meaning ratios of (sn1:sn2:sn3) 1:1.1:1, 1:1.5:1 to 1:3:1. Also, the arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid produced in the process likewise show, in the lipids and/or oils, a preference for the sn2-position in the triglyceride in 50

comparison with the positions sn1 and sn3 of advantageously 1:1.1:1, 1:1.5:1 to 1:3:1.

As described above, the polyunsaturated C20- and/or C_{22} -fatty acids, produced in the process, with four, five or six double bonds in the molecule will in the seed of plants which comprise no, or only very small amounts, of C12:0or C14:0-fatty acids. Even shorter saturated fatty acids such as the fatty acids C4:0, C6:0, C8:0 or C10:0, too, should not be present in the lipid and/or oil, or only in small amounts. Only small amounts are understood as meaning, advantageously, amounts which, when analyzed by GC, advantageously amount to less than 5, 4, 3, 2 or 1%, advantageously less than 0.9, 0.8, 0.7, 0.6 or 0.5%, especially advantageously less than 0.4, 0.3, 0.2 or 0.1%, very especially preferably less than 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02 or 0.01 units GC peak area. The fatty acid C16:0 should advantageously be in the range of from 1 to 28% units GC peak area. Advantageously, the fatty acid C16:0 should be present in amounts of less than 25%, 20%, 15% or 10%, advantageously less than 9%, 8%, 7%, 6% or 5%, especially advantageously of less than 4%, 3%, 2% or 1% units GC peak area or not at all in the lipids, oils and/or free fatty acids. The fatty acid C16:1 should advantageously amount to less than 1, 0.5, 0.4, 0.3, 0.2 or 0.1%, especially advantageously 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02 or 0.01 units GC peak area. Very especially preferably, the fatty acid C16:1 should not be present in the oils and/or lipids produced in the process. The same applies to the fatty acids C15:0, C17:0, C16:1^{Δ 3} trans, C16:4^{Δ 4,7,10,13} and C18:5^{Δ 3,6}, 9,12,15. Besides oleic acid (C18:1^{Δ 9}), the isomers (C18:1^{Δ 7}, C18:1^{Δ 11}) may also be present in the lipids, oils or free fatty acids. Advantageously in amounts of less than 5%, 4%, 3%, 2% or 1%, measured as units GC peak area. Each of the fatty acids C20:0, C20:1, C24:0 and C24:1 should be present in a range of from 0 to 1%, 0 to 3% and 0 to 5% units GC peak area, respectively. Moreover, little dihomo-y-linolenic acid (=DGLA) in terms of units GC peak area should be detectable in the seed oil and/or seed lipid in the GC analysis. Little is understood as meaning less than 2, 1.9, 1.8, 1.7, 1.6 and 1.5%, advantageously less than 1.4, 1.3, 1.2, 1.1 or 1%, especially advantageously less than 0.9, 0.8, 0.7, 0.6, 0.5 or 0.4% in terms of units GC peak area.

In a preferred embodiment of the process, DGLA and ARA should be produced in a ratio of from 1:1 up to 1:100, 45 advantageously 1:2 up to 1:80, especially advantageously 1:3 up to 1:70, very especially preferably 1:5 up to 1:60.

In a further preferred embodiment of the process, DGLA and EPA should be produced in a ratio of from 1:1 up to 1:100, advantageously 1:2 up to 1:80, especially advantageously 1:3 up to 1:70, very especially preferably 1:5 up to 1:60.

The lipids, oils and/or free fatty acids produced in the process according to the invention should advantageously have a high content of unsaturated fatty acids, advantageously of polyunsaturated acids, of at least 30, 40 or 50% by weight, advantageously of at least 60, 70 or 80% by weight, based on the total fatty acid content in the seeds of the transgenic plants.

All saturated fatty acids together should advantageously only account for a small amount in the lipids, oils and/or free fatty acids, preferably used plants. In this context, a small amount is understood as meaning an amount of less than 15%, 14%, 13%, 12%, 11% or 10%, preferably less than 9%, 8%, 7% or 6% in units GC peak area.

Lipids, oils and/or free fatty acids produced in the process should advantageously have an erucic acid content of less than 2% by weight based on the total fatty acid content of the plant. Advantageously, no erucic acid should be present in the lipids and/or oils. Also, the content of saturated fatty acids C16:0 and/or C18:0 should advantageously be less than 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10% by weight, advantageously less than 9, 8, 7, 6 or 5% by weight, based 5 on the total fatty acid content of the lipids and/or oils. Also, longer fatty acids such as C20:0 or C22:1 should not be present at all or only in small amounts of advantageously less than 4, 3, 2 or 1% by weight, advantageously less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 or 0.1% by weight, based 10 on the total fatty acid content of the lipids and/or oils. Typically, no, or only small amounts, of C16:1 are present as fatty acid in the lipids and/or oils produced in the process according to the invention. Small amounts are advantageously understood as meaning fatty acid contents of less 15 than 4, 3, 2 or 1% by weight, advantageously less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 or 0.1% by weight, based on the total fatty acid content of the lipids and/or oils.

The oils, lipids, fatty acids or fatty acid mixtures according to the invention which are obtained after pressing are 20 referred to as what is known as crude oils. They still comprise all of the oil and/or lipid contents and also compounds which are soluble in these. Such compounds are the various tocopherols such as α -tocopherol, β -tocopherol, γ -tocopherol and/or δ -tocopherol or phytosterols such as 25 brassicasterol, campesterol, stigmasterol, β-sitosterol, sitostanol, Δ^5 -avenasterol, Δ^5 ,24-stigmastadienol, Δ^7 -stigmasternol or $\Delta^7\text{-avenasterol}.$ These compounds are present in a range of from 1 to 1000 mg/100 g, advantageously 10 to 800 mg/100 g of lipid or oil. Triterpenes such as germaniol, 30 amyrin, cycloartenol and others may also be present in these lipids and oils. These lipids and/or oils comprise the polyunsaturated fatty acids produced in the process, such as ARA, EPA and/or DHA, bound in polar and unpolar lipids such as phospholipids, for example phosphatidylcholine, 35 phosphatidylethanolamine, phosphatidiylinositol, phosphatidylserine, phosphatidylglycerol, galactolipids, monoglycerides, diglycerides or triglycerides, to mention but a few. Lysophospholipids may also be present in the lipids and/or oils. These components of the lipids and/or oils can be 40 separated from one another by suitable processes. Cholesterol is not present in these crude oils.

A further embodiment according to the invention is the use of the oil, lipid, fatty acids and/or the fatty acid composition in feedstuffs, foodstuffs, cosmetics or pharmaceu- 45 ticals. The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used in the manner with which the skilled worker is familiar for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin such as, for example, fish oils. Typical of such fish oils 50 short-chain fatty acids such as C12:0, C14:0, C14:1, branched C15:0, C15:0, C16:0 or C16:1. Polyunsaturated C16-fatty acids such as C16:2, C16:3 or C16:4, branched C17:0, C17:1, branched C18:0 and C19:0 and also C19:0 and C19:1 are also found in fish oil. Such fatty acids are 55 typical of fish oils and are only found rarely, or not at all, in vegetable oils. Economically relevant fish oils are, for example, anchovy oil, menhaden oil, tuna oil, sardine oil, herring oil, mackerel oil, whale oil and salmon oil. These lipids and/or oils of animal origin can be used for mixing 60 with the oils according to the invention in the form of crude oils, i.e. in the form of lipids and/or oils which have not yet been purified, or else various purified fractions may be used for mixing.

A further embodiment according to the invention is the 65 use of the oil, lipid, fatty acids and/or fatty acid compositions in feedstuffs, foodstuffs, cosmetics or pharmaceuticals.

The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used in the manner with which the skilled worker is familiar for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin such as, for example, fish oils. Again, these oils, lipids, fatty acids or fatty acid mixtures, which are composed of vegetable and animal constituents, may be used for the preparation of foodstuffs, feedstuffs, cosmetics or pharmaceuticals.

The term "oil", "lipid" or "fat" is understood as meaning a fatty acid mixture comprising unsaturated or saturated, preferably esterified, fatty acid(s). The oil, lipid or fat is preferably high in polyunsaturated free or, advantageously, esterified fatty acid(s), in particular linoleic acid, y-linolenic acid, dihomo- γ -linolenic acid, arachidonic acid, α -linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid. The amount of unsaturated esterified fatty acids preferably amounts to approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80%, 85% or more is even more preferred. For the analysis, the fatty acid content can, for example, 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. The content of the various fatty acids in the oil or fat can vary, in particular depending on the starting organism.

The polyunsaturated fatty acids with advantageously at least two double bonds which are produced in the process are, as described above, for example sphingolipids, phosphoglycerides, lipids, glycolipids, phospholipids, monoacylglycerol, diacylglycerol, triacylglycerol or other fatty acid esters.

Starting from the polyunsaturated fatty acids with advantageously at least five or six double bonds, which acids have been prepared in the process according to the invention, the polyunsaturated fatty acids which are present can be liberated for example via treatment with alkali, for example aqueous KOH or NaOH, or 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 via, for example, H_2SO_4 . The fatty acids can also be liberated directly without the above-described processing step.

Mosses and algae are the only known plant systems which produce substantial 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, while algae, organisms which are related to algae and a few fungi also accumulate substantial amounts of PUFAs in the triacylglycerol fraction.

This is why nucleic acid molecules which are isolated from such strains which also accumulate PUFAs in the triacylglycerol fraction are particularly advantageous for the process according to the invention and thus for the modification of the lipid and PUFA production system in a host, in particular plants such as oil crops, for example oilseed rape, canola, linseed, hemp, soybeans, sunflowers and borage. They can therefore be used advantageously in the process according to the invention.

After their introduction into a plant cell or plant, the nucleic acids used in the process can either be present on a separate plasmid or, advantageously, integrated into the genome of the host cell. In the case of integration into the genome, integration can be random or else be effected by recombination such that the native gene is replaced by the copy introduced, whereby the production of the desired compound by the cell is modulated, or by the use of a gene in trans, so that the gene is linked operably with a functional expression unit which comprises at least one sequence which ensures the expression of a gene and at least one sequence which ensures the polyadenylation of a functionally transcribed gene. The nucleic acids are advantageously introduced into the organisms via multiexpression cassettes or constructs for multiparallel expression, advantageously 10 into the plants for the multiparallel seed-specific expression of genes.

Naturally, the coexpression of a plurality of genes can be effected not only by introducing the genes on a shared recombinant nucleic acid construct. Rather, individual genes 15 can also be introduced separately-simultaneously or in succession, on a variety of constructs. In this case, the simultaneous presence in the plant which coexpresses all of the genes is ensured by using different selection markers. This plant can be the product of one or more transformation 20 procedures, or else be a hybridization product of plants comprising one or more of the genes.

Substrates which are advantageously suitable for the nucleic acids which are used in the process according to the invention and which encode polypeptides with ω 3-desatu- 25 rase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 12$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase and/or $\Delta 9$ -elongase activity and/or the further nucleic acids used, such as the nucleic acids which encode polypeptides of the fatty acid or lipid metabolism selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [=acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acvltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxi- 35 dase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously C_{16} -, C_{18} -, C_{20} - or C_{22} -fatty acids. The fatty adds converted as substrates in the process are preferably con- 40 verted in the form of their acyl-CoA esters and/or their phospholipid esters. It is advantageous to use, in the process, desaturases with specificity for the acyl-CoA esters. The advantage here is that a substitution between the phospholipid esters, which are generally the substrate of the desatu- 45 ration, and the acyl-CoA esters, can be dispensed with. Thus, a further enzyme step which, as has been shown, is limiting in some cases, can be dispensed with.

To produce the long-chain PUFAs according to the invention, the polyunsaturated C_{16} - or C_{18} -fatty acids must first be 50 desaturated by the enzymatic activity of a desaturase and subsequently be elongated by at least two carbon atoms via an elongase. After one elongation cycle, this enzyme activity gives $\mathrm{C}_{18}\text{-}$ or $\mathrm{C}_{20}\text{-}\text{fatty}$ acids and after two elongation cycles $\mathrm{C_{20}}\text{-}$ or $\mathrm{C_{22}}\text{-}\text{fatty}$ acids. The activity of the desaturases and ~55elongases used in the process according to the invention preferably leads to C118-, C20- and/or C22-fatty acids, advantageously with at least two double bonds in the fatty acid molecule, preferably with three, four, five or six double bonds, especially preferably to give C20- and/or C22-fatty 60 d) at least one nucleic acid sequence with the sequence acids with, at least three double bonds in the fatty acid molecule, preferably with three, four, five or six double bonds, very specially preferably with four, five or six double bonds in the molecule/Products of the process according to the invention which are especially preferred are arachidonic 65 acid, eicosapentaenoic 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 the fatty acids, oils, lipids or fats in the plants which are advantageously used is. for example, in general the seed or cell strata 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 can also take place in a tissue-specific manner in all the other parts of the plant, for example in epidermal cells or in the tubers.

Owing to the use of the nucleic acids according to the invention which encode a $\Delta 5$ -elongase, the polyunsaturated fatty acids produced in the process can be increased by at least 5%, preferably by at least 10%, especially preferably by at least 20%, very especially preferably by at least 50% in comparison with the wild type of the organisms which do not comprise the nucleic acids recombinantly.

In principle, the polyunsaturated fatty acids produced by the process according to the invention in the plants used in the process can be increased in two different ways. Either the pool of free polyunsaturated fatty acids and/or the content of the esterified polyunsaturated fatty acids produced via the process can be enlarged. Advantageously, the pool of esterified polyunsaturated fatty acids in the transgenic organisms is enlarged by the process according to the invention.

A further subject matter according to the invention are isolated nucleic acid sequences which encode polypeptides with $\Delta 5$ -elongase, the $\Delta 5$ -elongases encoded by the nucleic acid sequences converting $\mathrm{C}_{20}\text{-}\mathrm{fatty}$ acids having at least four double bonds in the fatty acid molecule; which are advantageously ultimately incorporated into diacylglycerides and/or triacylglycerides.

A further subject matter of the invention is thus an isolated nucleic acid sequence which encodes polypeptides with Δ 5-elongase and which has the sequence shown in SEQ ID NO: 197.

A further subject matter of the invention is an isolated nucleic acid sequence which encodes polypeptides with Δ 6-elongase activity and which has the sequence shown in SEQ ID NO: 199.

Yet a further subject matter of the invention is an isolated nucleic acid sequence which encodes polypeptides with $\Delta 6$ -desaturase activity and which has the sequence shown in SEQ ID NO: 201.

The subject matters of the invention likewise extend to a recombinant nucleic acid molecule comprising:

- a) one or more copies of a promoter which is active in plant cells, preferably in seed cells,
- b) at least one nucleic acid sequence with the sequence shown in SEQ ID NO: 193 or SEQ ID NO: 201 which encodes a $\Delta 6$ -desaturase activity,
- c) at least one nucleic acid sequence with the sequence shown in SEQ ID NO: 11 which encodes a Δ 5-desaturase activity,
- shown in SEQ ID NO: 27 or SEQ ID NO: 199 which encodes a $\Delta 6$ -elongase activity, and
- e) one or more copies of a terminator sequence.

recombinant abovementioned nucleic acid molecule.

Advantageously, an additional nucleic acid sequence with the sequence shown in SEQ ID NO: 195 and which encodes a $\Delta 12$ -desaturase may also advantageously be present in the

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In a further advantageous embodiment, an additional nucleic acid sequence with the sequence shown in SEQ ID NO: 197 and which encodes a Δ 5-elongase may also be present in the recombinant nucleic acid molecule.

Besides these abovementioned sequences, further biosyn- 5 thetic genes 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 acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty 10 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) may also be introduced into the recombinant 15 nucleic acid molecule.

These genes are by preference genes of the fatty acid or lipid metabolism selected from the group consisting of Δ 4-desaturase, Δ 8-desaturase, Δ 9-desaturase or Δ 9-elongase.

Yet a further subject matter of the invention are gene constructs which comprise the nucleic acid sequences SEQ ID NO: 11, SEQ ID NO: 27, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 according to the invention, the nucleic acid being function- 25 ally linked to one or more regulatory signals.

All of the nucleic acid sequences used in the process according to the invention are advantageously derived from a eukaryotic organism such as a plant, a microorganism such as an alga or an animal. By preference, the nucleic acid 30 sequences are derived from the order Salmoniformes, Xenopus or Ciona, algae such as Mantoniella, Crypthecodinium, Euglena or Ostreococcus, fungi such as the genus Phytophtora or from diatoms such as the genera Thalassiosira or Phaeodactylum.

The nucleic acid sequences used in the process which encode proteins with ω 3-desaturase, Δ 4-desaturase, Δ 5-desaturase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 9$ -desaturase, $\Delta 12$ desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase or $\Delta 9$ -elongase activity are advantageously introduced by themselves or by 40 preference in combination with an expression cassette (=nucleic acid construct) which the expression of the nucleic acids in a plant. More than one nucleic acid sequence of an enzymatic activity such as, for example, a $\Delta 12$ -desaturase, Δ 4-desaturase, Δ 5-desaturase, Δ 6-desaturase, Δ 5-elongase, 45 $\Delta 6$ -elongase and/or to 3-desaturase may be present in the nucleic acid construct.

For introduction into the plant, the nucleic acids used in the process are advantageously subjected to amplification and ligation in the known manner as described above.

A series of mechanisms exist which enable a modification of the $\Delta 12$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 4$ -desaturase, $\Delta 6$ -desaturase and/or $\omega 3$ -desaturase protein according to the invention and of the further proteins used in the process, such as the $\Delta 12$ -desaturase, $\Delta 9$ -elon- 55 gase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase or $\Delta 4$ -desaturase proteins, so that the yield, production and/or production efficiency of the advantageously polyunsaturated fatty acids in a plant, preferably in an oil crop plant, can be influenced directly as the result of this 60 modified protein: The number or activity of the $\Delta 12$ -desaturase, ω 3-desaturase, Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase or $\Delta 4$ -desaturase proteins or genes can be increased so that larger amounts of the gene products and thus ultimately larger 65 amounts of the compounds of the general formula I are produced. A de-novo synthesis in a plant which had lacked

the activity and ability to biosynthesize the compounds prior to the introduction of the gene(s) in question is also possible. The same also applies analogously to the combination with further desaturases or elongases or further enzymes from the fatty acid and lipid metabolism. Also, the use of different, divergent sequences, i.e. sequences which differ at the DNA sequence level, may be advantageous, or the use of promoters for gene expression which makes possible a different temporal gene expression, for example depending on the degree of maturity of a seed or oil-storing tissue.

By introducing a $\Delta 12$ -desaturase, $\omega 3$ -desaturase, Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase and/or Δ 4-desaturase gene into a plant alone or in combination with other genes into a cell may not only increase the biosynthetic flux towards the end product, but also increase the corresponding triacylglycerol composition or create it de novo. Likewise, the number or activity of other genes in the import of nutrients required for the biosynthesis of one or more fatty acids, oils, polar and/or 20 neutral lipids may 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 is increased further, as described hereinbelow. By optimizing the activity or increasing the number of one or more $\Delta 12$ -desaturase, ω 3-desaturase, Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase or Δ 4-desaturase genes which are involved in the biosynthesis of these compounds, or by destroying the activity of one or more genes which are involving in breaking down these compounds, it may be possible to increase the yield, production and/or production efficiency of fatty acid and lipid molecules from organisms and advantageously from plants.

The isolated nucleic acid molecules used in the process 35 according to the invention encode proteins or parts of these, the proteins or the individual protein or parts thereof comprising an amino acid sequence with sufficient homology with an amino acid sequence which is shown in the sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36; SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54; SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76; SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 184, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200 or SEQ ID NO: 202 so that the proteins or parts thereof retain a $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, Δ 5-elongase or Δ 4-desaturase activity. The proteins or parts thereof, which is/are encoded by the nucleic acid molecule(s), preferably still retain(s) its/their essential enzymatic activity and the ability of participating in the metabolism of compounds required in the formation of cell membranes or lipid bodies in organisms, advantageously in plants, or in the transport of molecules across these membranes. Advantageously, the proteins encoded by the nucleic

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acid molecules have at least approximately 50%, preferably at least approximately 60% and more preferably at least approximately 70%, 80% or 90% and most preferably at least approximately 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more 5 identity with the amino sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ 10 ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, 15 SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEO ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ 20 tylum, Cryphthecodinium, specifically from the genera and ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 184, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200 or SEQ ID NO: 202. For the purposes of the invention, homology or homologous is 25 understood as meaning identity or identical.

The homology was calculated over the entire amino acid or nucleic acid sequence region. A series of programs which are based on the various algorithms ere available for comparing different sequences. In this context, the algorithms of 30 Needleman and Wunsch or Smith and Waterman give especially reliable results. To carry out the sequence alignments, the program PileUp (J. Mol. Evolution, 25, 351=360, 1987, Higgins et ah, CABIOS, 5 1989:151-153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 35 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison Wis., USA 53711 (1991)], were used. The sequence homology values stated above as percentages were 40 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 align- 45 ments.

Essential enzymatic activity of the $\Delta 12$ -desaturase, ω 3-desaturase, Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase or Δ 4-desaturase used in the process according to the invention is understood 50 as meaning that, in comparison with the proteins/enzymes encoded by the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 55 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ 60 ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 65 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ

ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 and their derivatives retain at least an enzymatic activity of at least 10%, preferably 20%, especially preferably 30% and very especially 40% and can thus participate in the metabolism of compounds required in the synthesis of fatty acids, fatty acid esters such as diacylglycerides and/or triacylglycerides in an organism, advantageously a plant or plant cell, or in the transport of molecules across membranes, meaning C18-, C20- or C22carbon chains in the fatty acid molecule with double bonds at least two, advantageously three, four, five or six positions.

The nucleic acids which can be used advantageously 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, Mantoniella, Ostreococcus, Isochrysis, Aleurita, Muscarioides, Mortierella, Borago, Phaeodacspecies Oncorhynchus mykiss, Xenopus laevis, Ciona intestinalis, Thalassiosira pseudonona, Mantoniella squamata, Ostreococcus sp., Ostreococcus tauri, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricornutum, Caenorhabditis elegans or especially advantageously from Oncorhynchus mykiss, Euglena gracilis, Thalassiosira pseudonona or Crypthecodinium cohnii.

As an alternative, it is possible to use, in the process according to the invention, nucleotide sequences which encode a $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase or Δ 4-desaturase and which hybridize, advantageously under stringent conditions, with a nucleotide sequence as shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEO ID NO: 67, SEO ID NO: 69, SEO ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201.

The nucleic acid sequences used in the process are advantageously introduced in an expression cassette which enables the expression of the nucleic acids in organisms such as microorganisms or plants.

In this context, the nucleic acid sequences which encode 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 Δ 4-desaturase are advantageously linked functionally with one or more regulatory signals to increase gene expression. These regulatory sequences should enable the targeted expression of the genes and protein expression. For

example, this may mean, depending on the host plant, that the gene is expressed and/or overexpressed only after induction has taken place, or else that it is expressed and/or overexpressed immediately. For example, these regulatory sequences take the form of sequences to which inductors or 5 repressors bind and thus regulate the expression of the nucleic acid. In addition to these new regulatory sequences, or instead of these sequences, the natural regulation of these sequences may still be present before the actual structural genes and, if appropriate, may have been genetically modi- 10 fied in such a way that the natural regulation has been switched off and the expression of the genes enhanced. The expression cassette (=expression construct=gene construct) may, however, also be simpler in construction, that is to say no additional regulatory signals were inserted before the 15 nucleic acid sequence or its derivatives, and the natural promoter together with its regulation was not removed. Instead, the natural regulatory sequence was mutated in such a way that regulation no longer takes place and/or gene expression is enhanced. These modified promoters can be 20 placed before the natural gene in order to increase the activity either in the form of part-sequences (=promoter with parts of the nucleic acid sequences according to the invention) or else alone. Moreover, the gene construct can advantageously also comprise one or more what are known as 25 "enhancer sequences" in functional linkage with the promoter, and these enable an increased expression of the nucleic acid sequence. Also, it is possible to insert additional advantageous sequences at the 3' end of the DNA sequences, such as further regulatory elements or terminators. The 30 $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 4$ -desaturase, $\Delta 5$ -desaturase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 5$ -elongase, $\Delta 6$ -elongase and/or Δ 9-elongase genes can be present in the expression cassette (=gene construct) as one or more copies. Advantageously, only in each case one copy of the genes is 35 present in the expression cassette. This gene construct, or the gene constructs, can be 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 inserted in the genome. It is advantageous 40 for the insertion of further genes in the host genome when the genes to be expressed are present together in one gene construct.

In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the 45 gene expression of the genes which have been introduced, thus increasing it. Thus, enhancement of the regulatory elements can advantageously take place at the transcription level by using strong transcription Signals such as promoters and/or enhancers. Besides, however, an enhancement of the 50 translation is also possible, for example by improving the stability of the mRNA.

Advantageous regulatory sequences for the new process are present for example in promoters such as the plant promoters CaMV/35S [Franck et al., Cell 21 (1980) 285- 55 294], PRP 1 [Ward et al., Plant Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, nos or in the ubiquitin or phaseolin promoter. Also advantageous in this context are inducible promoters, such as the promoters described in EP-A-0 388 186 (benzylsulfonamide-inducible), Plant J. 2, 60 1992:397-404 (Gatz et al., tetracyclin-inducible), EP-A-0 335 528 (abscisic-acid-inducible) or WO 93/21334 (ethanolor cyclohexenol-inducible). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8, 1989, 65 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from *Glycine max* (Genbank accession No.

U87999) or the node-specific promoter described in EP-A-0 249 676. Especially advantageous promoters are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Very especially advantageous are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially advantageous promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in U.S. Pat. No. 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from Arobidopsis), U.S. Pat. No. 5,504,200 (phaseolin promoter from Phaseolus vulgaris), WO 91/13980 (Bce4 promoter from Brassica), by Baeumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for example for monocots: Ipt-2 or Ipt-1 promoter from barley (WO 95/15389) and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890.

In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890.

To obtain a particularly high PUFA content especially in transgenic plants, the PUFA biosynthesis genes should advantageously be expressed in a seed-specific manner in oilseed crops. To this end, it is possible to use-seed-specific promoters or those promoters which are active in the embryo and/or in the endosperm. In principle, seed-specific promoters can be isolated both from dicotyledonous and from monocotyledonous plants. Such advantageous promoters are detailed further above, for example the USP, Vicilin, Napin, Oleosin, Phaseolin, Bce4, LegB4, Lpt2, Ipt1, Amy32b, Amy 6-6, Aleurain or Bce4 promoter.

Moreover, chemically inducible promoters are also advantageously useful in the process according to the invention.

Further advantageous promoters which are advantageously suitable for expression in soybean are the promoters of the β -conglycinin α -subunit, of the β -conglycinin β -subunit, of the Kunitz trypsin inhibitor, of annexin, of glysinin, of albumin 2S, of legumin A1, of legumin A2 and that of BD30.

Especially advantageous promoters are the USP, LegB4, Fad3, SBP, DC-3 or cruciferin 820 promoter.

Advantageous regulatory sequences which are used for the expression of the nucleic acid sequences used in the process according to the invention are terminators for the expression advantageously in soybean are Leg2A3', Kti3', Phas3', BD30 3' or AlS3'.

Especially advantageous terminators are the A7T, OCS, LeB3T or cat terminator.

To ensure a stable integration of the biosynthetic genes in the transgenic plant over several generations, each of the nucleic acids used in the process and which encodes $\Delta 12$ desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 4$ -desaturase should, as described above, be under the control of its own promoter, preferably of a different promoter, since repeating sequence motifs can lead to instability of the T-DNA, or to recombination events. As described above, the gene construct can also comprise further genes which are to be introduced into the plant.

In this context, the regulatory sequences or factors used advantageously for the expression of the nucleic acids used in the process according to the invention can, as described 5 above, preferably have a positive effect on the gene expression of the genes introduced.

These advantageous vectors; preferably expression vectors, comprise the nucleic acids used in the process which encode the $\Delta 12$ -desaturases, $\omega 3$ -desaturases, $\Delta 9$ -elongases, 10 $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\Delta 5$ -elongases or $\Delta 4$ -desaturases, or a nucleic acid construct which the used nucleic acid alone or in combination with further biosynthesis genes of the fatty acid or lipid metabolism such as the acyl-CoA:lysophospholipid acyltransferases, $\omega 3$ -desaturases, $\Delta 4$ -desaturases, $\Delta 5$ -desaturases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 9$ -desaturases, $\Delta 12$ desaturases, $\omega 3$ -desaturases, $\Delta 5$ -elongases, $\Delta 6$ -elongases and/or $\Delta 9$ -elongases.

As described and used in the present context, the term 20 "vector" refers to a nucleic acid molecule which is capable of transporting another nucleic acid to which it is bound.

The recombinant expression vectors used can be designed for expressing $\Delta 12$ -desaturases, $\omega 3$ -desaturases, $\Delta 9$ -elongases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 6$ -elongases, 25 Δ 5-desaturases, Δ 5-elongases and/or Δ 4-desaturases in prokaryotic or eukaryotic cells. This is advantageous since, for the sake of simplicity, intermediate steps of the vector construction are frequently carried out in microorganisms. For example, the $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elon- 30 gase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 4$ -desaturase 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) "Foreign gene expression in 35 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, Ed., pp. 396-428: Academic Press: San Diego; and van den Hondel, C. A. M. J. J., & 40 Punt, P. J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J. F., et al., Ed., pp. 1-28, Cambridge University Press: Cambridge), algae (Falciatore et al., 1999, Marine Biotechnology. 1, 3:239-251), ciliates of the types: 45 Holotrichia, Peritrichia, Spirotrichia, Suctoria, Tetrahymena, Paramecium, Colpidium, Glaucoma, Platvophrva, Potomacus, Desaturaseudocohnilembus, Euplotes, Engelmaniella and Stylonychia, in particular the genus Stylonychia lemnae, using vectors following a transformation pro- 50 cess as described in WO 98/01572, and preferably in cells of multi-celled plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants" Plant Cell Rep.: 583-586; Plant Molecular 55 Biology and Biotechnology, C Press, Boca Raton, Fla., 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, Ed.: Kung and R. Wu, Academic Press (1993), 128-43; Potrykus, Annu. Rev. Plant 60 Physiol. Plant Molec. Biol. 42 (1991), 205-225 (and references cited therein)). Suitable host cells are furthermore discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). As an alternative, the recombinant expression vector 65 can be transcribed and translated in vitro, for example using T7-promoter regulatory sequences and T7-polymerase.

In most cases, the expression of proteins in prokaryotes, advantageously for the simple detection of the enzyme activity for example for detecting the desaturase or elongase activity, is performed using vectors comprising constitutive or inducible promoters which control the expression of fusion or nonfusion proteins. Examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith, D. B., and Johnson, K. S. (1988) Gene 67:31-40), pMAL (New England Labs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.), where glutathione S-transferase (GST), maltose-E-binding protein and protein A, respectively, are fused with the recombinant target protein.

Examples of suitable inducible nonfusion *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, Calif. (1990) 60-89). The target gene expression of the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by host RNA polymerase. The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7gn1). 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 the transcriptional control of the lacUV 5 promoter.

The skilled worker is familiar with other vectors which are suitable in prokaryotic organisms, these vectors are, for example *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 pBdCl, 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 comprise 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, Calif.). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which 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., Ed. pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi [J. W. Bennett & L. L. Lasure, Ed., pp. 396-428: Academic Press: San Diego]. Further suitable yeast vectors are, for example, pAG-1, YEp6, YEp13 or pEMBLYe23.

As an alternative, the $\Delta 12$ -desaturases, u)-3-desaturases, $\Delta 9$ -elongases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\Delta 5$ -elongases and/or $\Delta 4$ -desaturases can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise 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 abovementioned vectors are only a small overview of possible suitable vectors. Further plasmids are known to the skilled worker and are described, for example, in: Cloning Vectors (Ed., 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 the 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, N.Y., 1989.

To detect the enzyme activity, $\Delta 12$ -desaturases, u)-3desaturases, $\Delta 9$ -elongases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, Δ 6-elongases, Δ 5-desaturases, Δ 5-elongases and/or Δ 4-desaturases can be expressed in single-cell plant cells (such as algae), see Falciatore et al., 1999, Marine Biotechnology 1 10 (3):239-251 and the references cited therein, and 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) "New plant 15 binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1195-1197; and 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, Vol. 20 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 controlling the gene expression in plant cells and which are functionally linked 25 so that each sequence can fulfill 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 the gene 3 of the Ti plasmid pTiACH5, which is known 30 as octopine synthase (Gielen et al., EMBO J. 3 (1984) 835 et seq.) or functional equivalents of these, but all other terminators which are functionally active in plants are also suitable.

Since plant gene expression is very often not limited to 35 transcriptional levels, a plant expression cassette preferably comprises other functionally linked sequences such as translation enhancers, for example the overdrive sequence, which comprises the 5'-untranslated tobacco mosaic virus leader sequence, which increases the protein/RNA ratio (Gallie et 40 al., 1987, Nucl. Acids Research 15:8693-8711).

As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissuespecific manner. Promoters which can be used are constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202) such as those which are derived from plant viruses such as 35S CAMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (see also U.S. Pat. No. 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small 50 subunit, which is described in U.S. Pat. No. 4,962,028.

Other preferred sequences for the use in functional linkage in plant gene expression cassettes are targeting sequences which are required for targeting the gene product into its relevant cell compartment (for a review, see Ker-55 mode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423 and references cited therein), for example into the vacuole, the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and 60 other compartments of plant cells.

As described above, plant gene expression can also be facilitated via a chemically inducible promoter (for a review, see Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly 65 suitable if it is desired that genes are expressed 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 promoters, for example the pathogen-inducible PRP1-gene promoter (Ward et al., Plant Mol. Biol. 22 (1993) 361-366), the heat-inducible hsp80 promoter from tomato (U.S. Pat. No. 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

The promoters which are especially preferred are those which bring about the expression of genes in tissues and organs in which fatty acid, lipid and oil biosynthesis takes place, in seed cells such as the cells of endosperm and of the developing embryo. Suitable promoters are the napin gene promoters from oilseed rape (U.S. Pat. No. 5,608,152), the USP promoter from Vicia faba (Baeumlein et al., Mol. Gen. Genet, 1991, 225 (3):459-67), the oleosin promoter from Arabidopsis (WO 98/45461), the phaseolin promoter from Phaseolus vulgaris (U.S. Pat. No. 5,504,200), the Bce4 promoter from Brassica (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seedspecific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable promoters to be taken into consideration are the Ipt2 or Ipt1 gene promoter from barley (WO 95/15389 and WO 95/23230) or those which are described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene).

In particular, the multiparallel expression of the $\Delta 12$ desaturases, $\omega 3$ -desaturases, $\Delta 9$ -elongases, $\Delta 6$ -desaturases, $\Delta 8$ -desaturases, $\Delta 6$ -elongases, $\Delta 5$ -desaturases, $\Delta 5$ -elongases and/or $\Delta 4$ -desaturases may be desired. Such expression cassettes can be introduced via a simultaneous transformation of a plurality of individual expression constructs or, preferably, by combining a plurality of expression cassettes on one construct. Also, it is possible to transform a plurality of vectors with in each case a plurality of expression cassettes and to transfer them to the host cell.

Likewise especially suitable are promoters which bring about the plastid-specific expression since plastids are the compartment in which the 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 clpP promoter from *Arabidopsis*, described in WO 99/46394.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction, as used in the present context, are intended to comprise a multiplicity, of prior-art processes for introducing foreign-nucleic acid (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, 2^{nd} ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Gold Spring Harbor, N.Y., 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, *Agrobacterium* protocols, Ed.: Gartland and Davey, Humana Press, Totowa, N.J.

The host organisms which are advantageously used are plant cells, preferably plants or parts thereof. Especially 5 preferred plants are plants such as oilseed plants or oil crops, which comprise large amounts of lipid compounds, such as oilseed rape, evening primrose, hemp, thistle, peanut, canola, linseed, soybean, safflower, Indian mustard, sunflower, borage or plants such as maize, wheat, rye, oats, 10 triticale, rice, barley, cotton, cassava, pepper, *Tagetes*, Solanaceae plants such as potato, tobacco, eggplant and tomato, *Vicia* species, pea, alfalfa, bushy plants (coffee, cacao, tea), *Salix* species, trees (oil palm, coconut) and perennial grasses and fodder crops. Especially preferred plants according to 15 the invention are oil crops such as soybean, peanut, oilseed rape, canola, linseed, hemp, evening primrose, sunflower, safflower, trees (oil palm, coconut).

As described above, a further subject matter according to the invention is an isolated nucleic acid sequence which 20 encodes polypeptides with $\Delta 5$ -elongase activity and which has the sequence shown in SEQ ID NO: 197, where the elongase encoded by the nucleic acid sequence does not elongate C₁₆- and C₁₈-fatty acids with one double bond. Polyunsaturated C₁₈-fatty acids with one $\Delta 6$ -double bond, or 25 C₂₂-fatty acids, are not converted either. Advantageously, only polyunsaturated C₂₀-fatty acids with one $\Delta 5$ -double bond are elongated by the enzymatic activity. Further subject matters of the invention are, as described above, a $\Delta 6$ -elongase, $\Delta 6$ -desaturase and a $\Delta 12$ -desaturase. 30

In an advantageous embodiment, the term "nucleic acid (molecule)" as used in the present text additionally comprises the untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, especially preferably 100 nucleotides of the sequence 35 upstream of the 5' terminus of the coding region and at least 100, preferably 50, especially preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. An "isolated" nucleic acid molecule is separated from other nucleic acid molecules which are present in 40 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 (for example sequences which are located at the 5' and 3' termini of the nucleic acid). 45 In various embodiments, the isolated $\Delta 12$ -desaturase, ω 3-desaturase, Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase or Δ 4-desaturase molecule can, for example, comprise less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 50 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, for example a nucleic acid molecule with a nucleotide sequence 55 of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID OO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, 65 SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, 66

SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 or part thereof, can be isolated using standard techniques of molecular biology and the sequence information provided herein. Also, for example a homologous sequence or homologous, conserved sequence regions at the DNA or amino acid level can be identified with the aid of comparative algorithms. These sequence regions can be used as hybridization probe and standard hybridization techniques (such as, for example, described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) for isolating further nucleic acid sequences which are useful in the process. Moreover, a nucleic acid molecule comprising a complete sequence of SEO ID NO: 1, SEO ID NO: 3, SEO ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO; 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97. SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 or part thereof can be isolated by polymerase chain reaction, where oligonucleotide primers which on the basis of this sequence or parts thereof are used (for example, a nucleic acid molecule comprising the complete sequence or part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been generated on the basis of this very sequence). For example, mRNA can be isolated from cells (for example by the guanidinium thiocyanate extraction process by Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA can be generated by means of reverse transcriptase (for example Moloney-MLV reverse transcriptase, from Gibco/BRL, Bethesda, Md., or AMV reverse transcriptase, from Seikagaku America, Inc., St. Petersburg, Fla.). Synthetic oligonucleotide primers for the amplification by means of polymerase chain reaction can be generated on the basis of one of the sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO:

101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 or with the aid of the amino acid 5 sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID 10 NO:34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ 15 ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEO ID NO: 104, SEO ID NO: 112, SEO ID 20 NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 184, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200 or SEQ ID NO: 202. One of the abovementioned nucleic acids can be amplified in accordance with 25 standard PCR amplification techniques using cDNA or, alternatively, genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified thus can be cloned into a suitable vector and characterized by means of DNA sequence analysis. Oligonucleotides which correspond 30 to a desaturase nucleotide sequence can be generated by synthetic standard methods, for example using an automatic DNA synthesizer.

Homologs of the $\Delta 12$ -desaturase, $\omega 3$ -desaturase, Δ 9-elongase, Δ 6-desaturase, Δ 8-desaturase, Δ 6-elongase, 35 Δ 5-desaturase, Δ 5-elongase or Δ 4-desaturase nucleic acid sequences used, with the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ 40 bp, 400 bp, 450 bp, 500 bp or more base pairs are used for ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 45 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEO ID NO: 73, SEO ID NO: 75, SEO ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ 50 ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201, mean for example allelic 55 variants with at least approximately 50 or 60%, preferably at least approximately 60 or 70%, more preferably at least approximately 70 or 80%, 90% or 95% and even more preferably at least approximately 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 60 99% or more identity or homology with one of the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 65 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO:

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39, SEQ ID NO: 41, SEQ ID NO; 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO; 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 or their homologs, derivatives or analogs or parts thereof. Furthermore, isolated nucleic acid molecules of a nucleotide sequence which hybridize, for example under stringent conditions, with one of the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEO ID NO: 21, SEO ID NO: 23, SEO ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201 or a part thereof. A part in accordance with the invention is understood as meaning, in this context, 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, especially preferably 350 the hybridization. Advantageously, the entire sequence may also be used. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from/into the sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO; 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 7-9, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 9T, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201, the intention being, however, that the enzyme activity of the resulting protein synthesized advantageously being retained for the insertion of one or more genes. Proteins which still retain the enzymatic activity of $\Delta 12$ desaturase, ω 3-desaturase, Δ 9-elongase, Δ 6-desaturase,

 Δ 8-desaturase, Δ 6-elongase, Δ 5-desaturase, Δ 5-elongase or Δ 4-desaturase, i.e. whose activity is essentially not reduced, mean proteins with at least 10%, preferably 20%, especially preferably 30%, very especially preferably 40% of the original enzyme activity in comparison with the protein 5 encoded by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO; 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, 10 SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID 15 NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEO ID NO: 111, SEO ID NO: 113, SEO ID 20 especially suitable for the production of PUFAs, preferably NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 183, SEQ ID NO: 193, SEQ ID NO: 197, SEQ ID NO: 199 or SEQ ID NO: 201. The homology was calculated over the entire amino acid or nucleic acid sequence region. A series 25 of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In this context, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. To carry out the sequence alignments, the program PileUp (J. Mol. Evo- 30 lution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 (1989:151-153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are part of the GCG software packet [Genetics 35 Computer Group, 575 Science Drive, Madison Wis., USA 53711 (1991)], were used. The sequence homology values detailed above in percent were determined using the program GAP over the entire sequence region with the following settings: Gap Weight: 50, Length Weight: 3, Average 40 Match: 10.000 and Average Mismatch: 0.000, which, unless otherwise specified, were always used as standard settings for sequence alignments.

Homologs of the abovementioned nucleic acid sequences also mean for example bacterial, fungal and plant homologs, 45 truncated sequences, single-stranded DNA or RNA of the coding and noncoding DNA sequence or else derivatives such as, for example, promoter variants. The promoters upstream of the nucleotide sequences stated can be modified by one or more nucleotide substitutions, by insertion(s) 50 and/or deletion(s), without, however, the functionality or activity of the promoters being adversely affected. Furthermore, it is possible that the activity of the promoters is increased by modifying their sequence, or that they are replaced completely by more active promoters, including 55 those from heterologous organisms.

The abovementioned nucleic acids and protein molecules with $\Delta 12$ -desaturase, $\omega 3$ -desaturase, $\Delta 9$ -elongase, $\Delta 6$ -desaturase, $\Delta 8$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, $\Delta 5$ -elongase and/or $\Delta 4$ -desaturase activity which are 60 involved in the metabolism of lipids and fatty acids, PUFA cofactors and enzymes or in the transport of lipophilic compounds across membranes are used in the process according to the invention for modulating the production of PUFAs in transgenic plants such as maize, wheat, rye, oats, 65 triticale, rice, barley, soybean, peanut, cotton, Linum species such as linseed or flax, Brassica species such as oilseed rape,

canola, Indian mustard and turnip rape, pepper, sunflower, borage, evening primrose and Tagetes, Solanaceae plants such as potato, tobacco, eggplant or tomato, Vicia species, pea, cassava, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut) and perennial grasses and fodder crops either directly (for example when the overexpression or optimization of a fatty acid biosynthetic protein has a direct effect on the yield, production and/or production efficiency of the fatty acid from modified organisms) and/or can have an indirect effect which nevertheless entails an increase in the yield, production and/or production efficiency of the PUFAs or a decrease of undesired compounds (for example when the modulation of the metabolism of lipids and fatty acids, cofactors and enzymes results in changes in the yield, production and/or production efficiency or the composition of the desired compounds within the cells which, in turn, can have an effect on the production of one or more fatty acids).

Brassicaceae, Boraginaceae, Primulaceae or Linaceae are of arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid. Especially suitable for the production of PUFAs with the nucleic acid sequences according to the invention, advantageously, as described, in combination with further desaturases and elongases are Indian mustard (Brassica juncea), oilseed rape and Camelina sativa.

The combination of a variety of precursor molecules and biosynthetic enzymes leads to the production of different fatty acid molecules, which has a major effect on the composition of the lipids since polyunsaturated fatty acids (=PUFAs) are incorporated not only into triacylglycerol but also into membrane lipids.

Brassicaceae, Boraginaceae, Primulaceae or Linaceae are especially suitable for the production of PUFAs, for example stearidonic acid, eicosapentaenoic acid or docosahexaenoic acid. Linseed (Linum usitatissumum) and Brassica juncea and Camelina sativa are especially advantageously suitable for the production of PUFAs with the nucleic acid sequences according to the invention, advantageously, as described, in combination with further desaturates and elongases.

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

Examples of precursors for PUFA biosynthesis are oleic acid, linoleic acid and linolenic acid. These C₁₈-carbon fatty acids must be elongated to C20 and C22 to obtain fatty acids 5 of the eicosa and docosa chain type. It is possible, with the aid of the desaturases used in the process, such as the $\Delta 12$ -, ω 3-, Δ 4-, Δ 5-, Δ 6- and Δ 8-desaturases and/or the Δ 5-, Δ 6-, $\Delta 9$ -elongases to produce arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid, 10 advantageously eicosapentaenoic acid and/or docosahexaenoic acid, and subsequently to use them for a variety of purposes in applications in the fields of foodstuffs, feedstuffs, cosmetics or pharmaceuticals. Using the abovementioned enzymes, C20- and/or C22-fatty acids with at least 15 two, advantageously at least three, four, five or six double bonds in the fatty acid molecule, preferably C20- or C22-fatty acids with advantageously four, five or six double bonds in the fatty acid molecule can be produced. The desaturation can take place before or after elongation of the fatty acid in 20 question. This is why the products of the desaturase activities and the further possible desaturation and elongation lead to preferred PUFAs with a higher degree of desaturation, including a further elongation of C20- to C22-fatty acids, to fatty acids such as γ-linolenic acid, dihomo-γ-linolenic acid, 25 arachidonic acid, stearidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. Substrates of the desaturases and elongases used in the process according to the invention are $\mathrm{C_{16}}\text{-},\,\mathrm{C_{18}}\text{-}$ or $\mathrm{C_{20}}\text{-}\text{fatty}$ acids such as, for example, linoleic acid, γ -linolenic acid, α -linolenic acid, dihomo- γ -linolenic 30 acid, eicosatetraenoic acid or stearidonic acid. Preferred substrates are linoleic acid, y-linolenic acid and/or a-linolenic acid, dihomo-y-linolenic acid or arachidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. The synthesized C20- to C22-fatty acids with at least two, three, four, 35 five or six, advantageously at least four, five or six double bonds in the fatty acid are obtained in the process according to the invention in the form of the free fatty acid or in the form of its esters, for example in the form of its glycerides.

The term "glyceride" is understood as meaning glycerol 40 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 or glyceride mixture can comprise further additions, for example free fatty acids, antioxidants, proteins, carbohydrates, vita- 45 mins 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 50 glycerophospholipids and glyceroglycolipids. Preferred examples which may be mentioned here are the glycerophospholipids such as lecithin (phosphatidylcholine), cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylaeylglycerophospholipids.

Furthermore, fatty acids must subsequently be transported to various sites of modification and incorporated into the triacylglycerol storage lipid. A further important step in lipid synthesis is the transfer of fatty acids onto the polar head groups, for example by glycerol-fatty-acid acyltransferase 60 (see Frentzen, 1998, Lipid, 100(4-5):161-166).

Publications on plant fatty acid biosynthesis, desaturation, the lipid metabolism and the transmembrane transport of fatty compounds, beta-oxidation, fatty acid modification and cofactors, triacylglycerol storage and assembly, including 65 the references therein, see the following articles: Kinney, 1997, Genetic Engineering, Ed., J K 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.: J K 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; Stymme et al., 1993, in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Ed.: Murata and Somerville, Rockville, American Society of Plant Physiologists, 150-158, Murphy & Ross 1998, Plant Journal. 13(1):1-16.

The PUFAs produced in the process comprise a group of molecules which higher animals are no longer capable of synthesizing and must therefore take up, or which higher animals are no longer capable of synthesizing themselves in sufficient quantity and must therefore take up additionally, although they can be readily synthesized by other organisms such as bacteria; for example, cats are no longer capable of synthesizing arachidonic acid.

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

The terms "production" or "productivity" are known in the art and refer to the concentration of the fermentation product (compounds of the formula I) formed within a certain period of time and a certain fermentation volume (for example kg of product per hour per liter). They also encompass the productivity within a plant cell or a plant, i.e. the content of the desired fatty acids produced in the process based on the content of all fatty acids in this cell or plant. The term production efficiency encompasses the time required for obtaining a certain amount of product (for example the time required by the cell for establishing a certain throughput rate of a fine chemical). The term "yield" or "product/carbon yield" is known in the art and comprises the efficiency of the conversion of the carbon source into the product (i.e. the fine chemical). This is usually expressed for example as kg of product per kg of carbon source. By increasing the yield or production of the compound, the amount of the obtained molecules or of the suitable obtained molecules of this compound in a certain amount of culture is increased over a specified period.

The terms "biosynthesis" or "biosynthetic pathway" are known in the art and comprise the synthesis of a compound, preferably of an organic compound, by a cell starting from intermediates, for example in a multistep process which is highly regulated. The terms "catabolism" or "catabolic pathway" are known in the art and comprise the cleavage of a compound, preferably of an organic compound, by a cell to give catabolytes (in more general terms, smaller or less complex molecules), for example in a multistep process which is highly regulated.

The term "metabolism" is known in the art and encompasses the totality of the biochemical reactions which take place in an organism. Thus, the metabolism of a certain compound (for example the metabolism of a fatty acid) comprises the totality of the biosynthetic, modification and catabolic pathways of this compound in the cell.

This 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.

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EXAMPLES

Example 1

General Cloning Methods

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA 74

Example 4

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

To clone the two sequences for heterologous expression in yeasts, the following oligonucleotides were used for the PCR reaction:

Pr	ime	r		Nucleotide sequence				
5'	f*	OmELO2	5'	aagcttacataatggcttcaacatggcaa	(SEQ	ID	NO :	179)
3'	r*	OmELO2	5'	ggatcettatgtettettgetetteetgtt	(SEQ	ID	NO :	180)
5'	f	OmELO3	5'	aagcttacataatggagacttttaat	(SEQ	ID	NO :	181)
3'	r	OmELO3	5'	ggatcettcagtcccccctcactttcc	(SEQ	ID	NO :	182)

*f: forward, r: reverse

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fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of E. 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 process of Sanger (Sanger et al.: (1977) Proc. Natl. Acad. Sci. USA74, 35 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 Genes from Oncorhynchus mykiss

As the result of a search for conserved regions in the protein sequences corresponding to the elongase genes detailed in the application, two sequences with suitable motifs were identified in the Genbank sequence database.

Name of gene	Genbank No.	Amino acids
OmELO2	CA385234, CA364848, CA366480	264
OmELO3	CA360014, CA350786	295

Total RNA from Oncorhynchus mykiss was isolated with 55 the aid of the RNAeasy Kit from Qiagen (Valencia, Calif., US). Poly-A+ RNA (mRNA) was isolated from the total RNA with the aid of oligo-dT cellulose (Sambrook et al., 1989). The RNA was subjected to reverse transcription using 60 the reverse transcription system kit from Promega, and the cDNA synthesized was cloned into the lambda ZAP vector (lambda ZAP Gold, Stratagene). The cDNA was depackaged in accordance with the manufacturer's instructions to give the plasmid DNA. The cDNA plasmid library was then used for the PCR for cloning expression plasmids.

Composition of the PCR Mix (50 µl):

- 5.00 µl template cDNA
- 5.00 µl 10× buffer (Advantage polymerase)+25 mM MgCl₂
- 25 5.00 µl of 2 mM dNTP
 - 1.25 μ l of each primer (10 pmol/ μ l)
 - 0.50 µl of Advantage polymerase (Clontech)
 - PCR Reaction Conditions:
- 30 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 enzymes HindIII and BamHI. The yeast expression vector pYES3 (Invitrogen) was incubated in the same manner. Thereafter, the 812 bp PCR product and the 905 bp PCR product and the vector were separated by 40 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, the vector and the elongase cDNA were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids pYES3-Om-ELO2 and pYES3-OmELO3 were verified by sequencing and transformed into the Saccharomyces strain INVSc1 (Invitrogen) by means of electroporation (1500 V). As a control, pYES3 was transformed in parallel. Thereafter, the 50 yeasts were plated onto complete tryptophan dropout minimal medium supplement with 2% glucose. Cells which are capable of growing on without tryptophan in the medium thus comprise the corresponding plasmids pYES3, pYES3-OmELO2 (SEQ ID NO: 51) and pYES3-OmELO3 (SEQ ID NO: 53). After the selection, in each case two transformants were selected for the further functional expression.

Example 5

Cloning Expression Plasmids for the Seed-Specific Expression in Plants

To transform plants, a further transformation vector based 65 on pSUN-USP was generated. To this end, NotI cleavage sites were introduced at the 5' and 3' termini of the coding sequence using the following primer pair:

PSUN-OmEL	02
	(SEQ ID NO: 175)
Forward:	5 ' - GCGGCCGCATAATGGCTTCAACATGGCAA
	(SEQ ID NO: 176)
Reverse:	3 ' - GCGGCCGCTTATGTCTTCTTGCTCTTCCTGTT
PSUN-OmEL	.03
	(SEQ ID NO: 177)
Forward:	5'-GCGGCCGCataatggagacttttaat
	(SEQ ID NO: 178)
Reverse:	3'-GCGGCCGCtcagtccccctcactttcc

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 5.00 µl of 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 ul 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 products were incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP was incubated in the same manner. 25 Thereafter, the PCR products and the 7624 bp 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 30 and PCR products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids pSUN-OmELO2 and pSUN-OmELO3 were verified by sequencing.

pSUN300 is a derivative of the plasmid pPZP (Hajduk- 35 iewicz P., Svab, Z, Maliga P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol. Biol. 25:989-994). pSUN-USP originated from pSUN300 by inserting a USP promoter as EcoRI fragment into pSUN 300. The polyadenylation signal 40 is that of the octopin synthase gene from the A. tumefaciens Ti plasmid (ocs terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid- 45 encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). The USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), part of the noncoding region of the USP gene being present in the promoter. The promoter fragment, which is 684 base pairs in 50 size, was amplified via a PCR reaction by standard methods, by means of commercially available T7 standard primer (Stratagene) and with the aid of a synthesized primer (primer sequence: 5'-GTCGACCGGCGGACTAGTGGGC-CCTCTAGACCCGGGGGGATCC GGATCTGCTTGGC- 55 TATGAA-3', SEQ ID NO: 174). The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid named pSUN-USP. The construct was used for transforming Ara-60 bidopsis thaliana, oilseed rape, tobacco and linseed.

Example 6

Lipid Extraction from Yeasts and Seeds

The effect of the genetic modification in plants, fungi, algae, ciliates or on the production of a desired compound

(such as a fatty acid) can be determined by growing the modified microorganisms or 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 chro-20 matography, various types of staining methods, enzymatic and microbiological methods and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", p. 469-714, VCH: Weinheim; Belter, P. A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J. F., and Cabral, J. M. S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J. A., and Henry, J. D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. B3; Chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F. J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned methods, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22): 12935-12940 and Browse et al: (1986) Analytic Biochemistry 152:141-145. The qualitative and quantitative analysis of lipids or fatty acids is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide-Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952)-16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

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

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

The unambiguous detection for the presence of fatty acid 5 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; 10 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 15 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% dimethoxy- 20 propane, 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) 25 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 35 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 40 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 45 (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-dimethoxyoxazolin derivatives (Christie, 1998) by means of GC-MS.

Yeasts which had been transformed with the plasmids 50 pYES3, pYES3-OmELO2 and pYES3-OmELO3 as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, 10 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 in order to remove residual medium 55 and fatty acids. Fatty acid methyl esters (FAMEs) were prepared with the yeast cell sediments by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. with 2 ml of 1N methanolic sulfuric acid and 2% (v/v) dimethoxypropane. The FAMEs were extracted by twice 60 extracting with petroleum ether (PE). To remove non-derivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0, and 2 ml of distilled water. Thereafter, the PE phases were dried with Na₂SO₄, evaporated under argon and taken up in 100 μ l of 65 PE. The samples were separated on a DB-23 capillary column (30 m, 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. with an increment of 5° C./min and finally 10 minutes at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding 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.

Example 7

Functional Characterization of OmELO2 and OmELO3

OmELO2 shows no elongase activity, while a pronounced, activity was detected for OmELO3, using different substrates. The substrate specificity of OmElo3 was determined after expression and feeding with various fatty acids (FIG. 2). The fed substrates can be detected in large amounts in all transgenic yeasts. All transgenic yeasts show that new fatty acids have been synthesized, to the products of the OmElo3 reaction. This means that the gene OmElo3 was expressed functionally.

FIG. 2 demonstrates that OmElo3 has a substrate specificity which leads to the elongation of $\Delta 5$ - and $\Delta 6$ -fatty acids with one w-double bond with high specificity. Moreover, $\omega 6$ -fatty acids (C18 and C20) were also elongated, with less specificity. The best substrates for OmElo3 were stearidonic acid (C18:4 ω 3) and eicosapentaenoic acid (C20:5 ω 3) (up to 66% elongation).

Example 8

Reconstitution of the Synthesis of DHA in Yeast

The reconstitution of the biosynthesis of DHA (22:6 ω 3) was carried out starting from EPA (20:5 ω 3) or stearidonic acid (18:4 ω 3) by coexpressing OmElo3 together with the Euglena gracilis $\Delta 4$ -desaturase or the Phaeodactylum tricornutum Δ 5-desaturase and the Euglena gracilis Δ 4-desaturase. To this end, the expression vectors pYes2-EgD4 and pESCLeu-PtD5 were additionally constructed. The abovementioned yeast strain which is already transformed with pYes3-OmElo3 (SEQ ID NO: 55), was then transformed further with pYes2-EgD4, or simultaneously with pYes2-EgD4 and pESCLeu-PtD5. The transformed yeasts were selected on complete minimal dropout tryptophan and uracil medium agar plates supplemented with 2% glucose in the case of the pYes3-pYes3-OmEIO/pYes2-EgD4 strain and complete minimal dropout tryptophan, uracil and leucine medium in the case of the pYes3-OmEIO/pYes2-EgD4+ pESCLeu-PtD5 strain. Expression was then induced by addition of 2% (w/v) galactose. The cultures were subsequently incubated for a further 120 hours at 15° C.

FIG. **3** shows the fatty acid profiles of transgenic yeasts which have been fed 20:5 ω 3. In the control yeast (A), which had been transformed with the vector pYes3-OmElo3 and the blank vector pYes2, 20:5 ω 3 was elongated highly efficiently to give 22:5 ω 3 (65% elongation). The additional introduction of the EEgA4-desaturase led to the conversion of 22:5 ω 3 into 22:6 ω 3 DHA. The fatty acid composition

of the transgenic yeasts is shown in FIG. **5**. After coexpression of OmElo3 and EgD4, up to 3% DHA was detected in yeasts.

In a further coexpression experiment, OmElo3, EgD4 and a Δ 5-desaturase from *P. tricomutum* (PtD5) were expressed 5 together. The transgenic yeasts were fed stearidonic acid (18:4 ω 3) and analyzed (FIG. 4). The fatty acid composition of these yeasts is shown in FIG. 5. OmElo3 elongated the fed fatty acid 18:4 ω 3 to give 20:4 ω 3 (60% elongation). The latter was desaturated by PtD5 to give 20:5 ω 3. The PtD5 10 activity amounted to 15%. Furthermore, 20:5 ω 3 was elongated by EmElo3 to give 22:5 ω 3. Thereafter, the newly synthesized 22:5 ω 3 was desaturated to give 22:6 ω 3 (DHA). Up to 0.7% of DHA was obtained in these experiments. 15

These experiments demonstrate that the sequences OmElo3, EgD4 and PtD5 which are used in the present invention are suitable for the production of DHA in eukaryotic cells.

Example 9

Generation of Transgenic Plants

a) Generation of Transgenic Oilseed Rape Plants (Modified 25 Process of Moloney et al., 1992, Plant Cell Reports, 8:238-242)

The binary vectors in Agrobacterium tumefaciens C58C1: pGV2260 or Escherichia coli (Deblaere et al, 1984, Nucl. Acids. Res. 13, 4777-4788) can be used for generating 30 transgenic oilseed rape plants. To transform oilseed rape plants (Var. Drakkar, NPZ Nordeutsche Pflanzenzucht, Hohenlieth, Germany), a 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Murashige and Skoog 1962 35 Physiol. Plant. 15, 473) supplemented with 3% sucrose (3MS medium) is used. Petiols or hypocotyls of freshly germinated sterile oilseed rape plants (in each case approx. 1 cm²) are incubated with a 1:50 agrobacterial dilution for 5-10 minutes in a petri dish. This is followed by 3 days of 40 coincubation in the dark at 25° C. on 3MS medium supplemented with 0.8% Bacto agar. The cultures are then grown for 3 days at 16 hours light/8 hours dark. The cultivation is then continued in a weekly rhythm on MS medium supplemented with 500 mg/l Claforan (cefotaxim sodium), 50 mg/l 45 ends). kanamycin, 20 µM benzylaminopurine (BAP), now supplemented with 1.6 g/l of glucose. Growing shoots are transferred to MS medium supplemented with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots have developed after three weeks, 2-indolebutyric acid is added to 50 the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium supplemented with kanamycin and Claforan; after rooting, they were transferred to compost and, after growing on for 80

two weeks in a controlled-environment cabinet or in the greenhouse, allowed to flower, and mature seeds were harvested and analyzed by lipid analysis for elongase expression, such as $\Delta 5$ -elongase or $\Delta 6$ -elongase activity. In this manner, lines with elevated contents of polyunsaturated C₂₀- and C₂₂-fatty acids can be identified.

b) Generation of Transgenic Linseed Plants

Transgenic linseed plants can be generated for example by the process of Bell et al., 1999, In Vitro Cell. Dev. Biol.-Plant. 35(6):456-465 by means of particle bombardment. Usually, an *agrobacteria*-mediated transformations was used for the transformation of linseed, for example by the process of Mlynarova et al. (1994), Plant Cell Report 13: 282-285.

Example 10

Cloning Δ5-Elongase Genes from *Thraustochytrium* aureum ATCC34304 and *Thraustochytrium* ssp

Comparisons of the various elongase protein sequences found in the present application enabled the definition of conserved nucleic acid regions (histidin box: His-Val-X-His-His, tyrosin box: Met-Tyr-X-Tyr-Tyr). An EST database of *T. aureum* ATCC34304 and *Thraustochytrium* ssp. was screened for further Δ 5-elongases with the aid of these sequences. The following new sequences were found:

Name of gene	Nucleotides	Amino acids
BioTaurELO1	828 bp	275
TL16y2	831	276

Total RNA from *T. aureum* ATCC34304 and *Thraus-tochytrium* ssp. was isolated with the aid of the RNAeasy Kits from Qiagen (Valencia, Calif., US). mRNA was isolated from the total RNA with the aid of the polyATract isolation system (Promega). The mRNA was subjected to reverse transcription using the Marathon cDNA Amplification Kit (BD Biosciences) and adaptors were ligated in accordance with the manufacturer's instructions. The cDNA library was then employed for the PCR for cloning expression plasmids by means of 5'- and 3'-RACE (rapid amplification of cDNA ends).

Example 11

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

To clone the sequence for heterologous expression in yeasts, the following oligonucleotides were used for the PCR reaction:

Pr	ime	r		Nucleotide sequence	2			
5'	f*	BioTaurEL01	5'	gacataatgacgagcaacatgag	(SEQ	ID	NO :	170)
3'	r*	BioTaurELO1	5'	cggcttaggccgacttggccttggg	(SEQ	ID	NO :	171)
5'	f*	TL16y2	5'	agacataatggacgtcgtcgagcagcaatg	(SEQ	ID	NO :	172)
3'	r*	TL16y2	5'	ttagatggtettetgettettgggegee	(SEQ	ID	NO :	173)

*f: forward, r: reverse

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Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

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

 $5.00 \ \mu l$ of $2 \ mM \ dNTP$

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.

Denaturation temperature: 1 min 94° C.

Elongation temperature: 2 min 72° C.

Number of cycles: 35

The PCR products BioTaurELO1 (see (SEQ ID NO: 65) and TL16v2 (see SEQ ID NO: 83) were incubated for 30 minutes at 21° C. with the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. The PCR product is ligated into the vector by means of a Toverhang and activity of a topoisomerase (Invitrogen). After incubation, E. coli DH5 α cells were transformed. 20 Suitable clones were identified by PCR, the plasmid DNA was isolated by means of Qiagen DNAeasy Kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector $_{25}$ pYES2.1 was transformed in parallel. The yeasts were subsequently plated onto complete uracil dropout minimal medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1, pYES2.1-BioTaurELO1 and pYES2.1-TL16y2. After the selection, in each case two transformants were selected for further functional expression.

Example 12

Cloning Expression Plasmids for the Seed Specific Expression in Plants

A further transformation vector based on pSUN-USP was generated for the transformation of plants. To this end, NotI cleavage sites were introduced at the 5' and 3' termini of the coding sequence, using the following primer pair:

PSUN-BioTaurELO1 Forward: (SEO ID NO: 166) 5'-GCGGCCGCATAATGACGAGCAACATGAGC 50 Reverse · (SEQ ID NO: 167) 3 ' - GCGGCCGCTTAGGCCGACTTGGCCTTGGG PSUN-TL16v2 -Forward: (SEQ ID NO: 168) 55 5 ' - GCGGCCGCACCATGGACGTCGTCGAGCAGCAATG Reverse: (SEO ID NO: 169) 5 ' - GCGGCCGCTTAGATGGTCTTCTGCTTCTTGGGCGCC Composition of the PCR Mix (50 µl): 5.00 µl template cDNA 5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂

5.00 µl 2 mM dNTP

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

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. 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 products were incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp 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 products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids pSUN-BioTaurELO1 and pSUN-TL16y2 were verified by sequenc-

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z; Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP

³⁵ gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene). (Primer sequence: 5'-GTCGAC-CCGCGGACTAGTGGGCCCTCTAGAC-

CCGGGGGGATCC GGATCTGCTGGCTATGAA-3', SEQ ID NO: 165). The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The 45 construct was used for the transformation of Arabidopsis

thaliana, oilseed rape, tobacco and linseed.

Lipids were extracted from yeasts and seeds as described for Example 6.

Example 13

Functional Characterization of BioTaurELO1 and TL16y2

The substrate specificity of BioTaurELO1 was determined after expression and feeding of various fatty acids (FIG. 6). FIG. 6 shows the feeding experiments for determining the functionality and substrate specificity with yeast strains comprising either the vector pYes2.1 (control) or the 60 vector pYes2.1-BioTaurELO1 (=BioTaur) with the Δ 5-elongase. In both approaches, 200 nm of y-linolenic acid and eicosapentaenoic acid were added to the yeast incubation medium and incubated for 24 hours. After the fatty acids had been extracted from the yeasts, they were transmethylated and separated by gas chromatography. The elongation products originating from the two fatty acids which had been fed are identified by arrows.

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The substrates which had been fed can be detected in large amounts in all transgenic yeasts. All transgenic yeasts-show that new fatty acids have been synthesized, the products of the BioTaurELO1 reaction; This means that the gene Bio-TaurELO1 has been expressed functionally.

FIG. **6** shows that BioTaurELO1 has a substrate specificity which leads with high specificity to the elongation of $\Delta 5$ and $\Delta 6$ -fatty acids with one ω -3-double bond. Moreover, $\omega 6$ -fatty acids (C18 and C20) were also elongated. γ -Linolenic acid (C18:3 $\omega 6$) is converted with a conversion rate of 65.28%, stearidonic acid (C18:4 $\omega 3$) with a conversion rate of 65.66% and eicosapentaenoic acid (C20:5 $\omega 3$) with a conversion rate of 22.01%. The substrate specificities of the various feeding experiments are shown in Table 6 (see end of the description).

The conversion rate of GLA when feeding GLA and EPA was 65.28%. The conversion rate of EPA, again when feeding GLA and EPA, was 9.99%. When only EPA was fed, the EPA conversion rate was 22.01%. Arachidonic acid (=ARA) was also converted when fed. The conversion rate 20 was 14.47%. Stearidonic acid (=SDA) was also converted. In this case, the conversion rate was 65.66%.

The functionality and substrate specificity of TL16y2 were determined after expression and feeding of various fatty acids. Table 7 shows the feeding experiments. The 25 feeding experiments were carried out in the same manner as described for BioTaurELO1. The substrates which have been fed can be detected in large amounts in all transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the TL16y2 reaction (FIG. 30 **11**). This means that the gene TL16y2 has been expressed functionally.

TABLE 7

bond. Then, C20-fatty acids with a $\Delta 5$ - or $\Delta 8$ -double bond are elongated, depending on the concentration of fatty acids which are fed.

Example 14

Cloning Genes from Ostreococcus tauri

The search for conserved regions in the protein sequences with the aid of the elongase genes with $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity which are shown in the application allowed the identification of sequences with suitable motifs in an *Ostreococcus tauri* sequence database (genomic sequences).

The sequences were the following:

Name of gene	SEQ ID	Amino acids
OtELO1, (Δ 5-elongase)	SEQ ID NO: 67	300
OtELO2, (Δ 6-elongase)	SEQ ID NO: 69	292

OtElo1 shows the highest similarity with an elongase from *Danio rerio* (GenBank AAN77156; identity approx. 26%), while OtElo2 shows the highest similarity with the *Physcomitrella* Elo (PSE) [approx. 36% identity] (alignments were carried out using the tBLASTn algorithm (Altschul et al., J. Mol. Biol. 1990, 215:403-410).

The cloning procedure was as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down, resuspended in 100 of doubledistilled water and stored at -20° C. The respective genomic

		%		ssion of T he gas-chi		yeast. phic analy	vsis		
Plasmid	Fatty acid	C18:3 (n-6)	C18:4 (n-3)	C20:3 (n-6)	C20:4 (n-6)	C20:4 (n-3)	C20:5 (n-3)	C22:4 (n-6)	C22:5 (n-3)
pYES	250 µm						13.79		
	EPA								
TL16y2	250 μm						25.81		2.25
	EPA								
pYES	50 µm						5.07		
	EPA								
TL16y2	50 μm						2.48		1.73
	EPA								
pYES	250 μm	8.31							
	GLA								
TL16y2	250 μm	3.59		10.71					
	GLA								
pYES	250 μm				16.03				
	ARA								
TL16y2					15.2		3.87		
MEG	ARA		26.70			0.25			
pYES	250 μm		26.79			0.35			
TT 1 ()	SDA					20.45			
TL16y2			7.74			29.17			
	SDA								

The results with TL16y2, which are shown in Table 7, show the following conversion rates in % of the control: a) $_{60}$ conversion rate of EPA in % (250 µm): 8%, b) conversion rate of EPA in % (50 µm): 41%; c) conversion rate of ARA in %: 20.3%, d) conversion rate of SDA in %: 79.4%, and e) conversion rate of GLA in %: 74.9%. $_{65}$

Thus, TL16y2 shows $\Delta 5$ -, $\Delta 6$ - and $\Delta 8$ -elongase activity. The activity is highest for C18-fatty acids with $\Delta 6$ -double

DNAs were amplified on the basis of the PCR process. The relevant primer pairs were selected in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the OtElo DNAs was carried out in each case using 1 μ l of defrosted cells, 200 μ m of dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 μ l. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 cycles

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of 30 seconds at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a last elongation step of 10 minutes at 72° C.

Example 15

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

To characterize the function of the Ostreococcus tauri elongases, the open reading frames of the DNAs in question 10were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to pOTE1 and pOTE2.

The Saccharomyces cerevisiae strain 334 was transformed by electroporation (1500 v) with the vector pOTE1 or pOTE2. A yeast which was transformed with the blank vector pYES2 was used as the control. The transformed yeasts were selected on complete minimal dropout uracil medium (CMdum) agar plates supplemented with 2% glu- $_{20}$ cose. After the selection, in each case three transformants were selected for the further functional expression.

To express the Ot elongases, precultures of in each case 5 ml of dropout uracil CMdum liquid medium supplemented with 2% (w/v) raffinose were inoculated with the selected 25 transformants and incubated for 2 days at 30° C., 200 rpm.

5 ml of CMdum liquid medium (without uracil) supplemented with 2% raffinose and 300 µm of various fatty acids were then inoculated with the precultures to an OD_{600} of 0.05. The expression was induced by addition of 2% (w/v) 30 galactose. The cultures were incubated for a further 96 hours at 20° C.

Example 16

Cloning of Expression Plasmids for the Seed-Specific Expression in Plants

A further transformation vector based on pSUN-USP was generated for the transformation of plants. To this end, NotI 40 cleavage sites were introduced at the 5' and 3' termini of the coding sequences, using PCR. The corresponding primer sequences are derived from the 5' and 3' regions of OtElo1 and OtElo2.

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

 $5.00 \,\mu\text{l}$ 10× buffer (Advantage polymerase)+25 mM MgCl₂

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. 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 products were incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the vector were separated 60 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 products were ligated. The Rapid Ligation Kit from Roche was used for 65 this purpose. The resulting plasmids pSUN-OtELO1 and pSUN-OtELO2 were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Ostreococcvs gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene). (Primer sequence: 5'-GTCGAC-CCGCGGACTAGTGGGCCCTCTAGAC-

CCGGGGGGATCC GGATCTGCTGGCTATGAA-3', SEQ ID NO: 164).

The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 17

Expression of OtELO1 and OtELO2 in Yeasts

Yeasts which had been transformed with the plasmids pYES3, pYES3-OtELO1 and pYES3-OtELO2 as described in Example 15 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour 45 at 80° C. together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were 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 equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50° C. to 250° C. with an increment of 5° C./min and finally 10 min at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding 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.

Example 18

Functional Characterization of OtELO1 and OtELO2

The substrate specificity of OtELo1 could be determined after expression and the feeding of different fatty acids (Tab. 8). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated 10the synthesis of novel fatty acids, the products of the OtElo1 reaction. This means that the gene OtElo1 has been expressed functionally.

It can be seen from Table 7 that OtElo1 has a narrow substrate specificity. OtElo1 was only capable of elongating the C20-fatty acids eicosapentaenoic acid (FIG. 7) and arachidonic acid (FIG. 8), but preferred the ω 3-desaturated eicosapentaenoic acid.

TABLE 8

 IA	BLE 8	20
Fatty acid substrate	Conversion rate (in %)	
16:0	_	
16:1 ^{Δ9}	_	
18:0	_	
$18:1^{\Delta 9}$	_	25
$18:1^{\Delta 11}$	_	
$18:2^{\Delta 9,12}$	_	
$18:3^{\Delta 6,9,12}$	_	
$18:3^{\Delta 5,9,12}$	_	
$20:3^{\Delta 8,11,14}$	_	
$20:4^{\Delta 5,8,11,14}$	10.8 ± 0.6	30
$20:5^{\Delta 5,8,11,14,17}$	46.8 ± 3.6	50
$22:4^{\Delta7,10,13,16}$	_	
22:6 ^{Δ4,7,10,13,16,19}	—	

Table 8 shows the substrate specificity of the elongase 35 OtElo1 for C20-polyunsaturated fatty acids with one double bond in Δ 5-position in comparison with various fatty acids.

The yeasts which had been transformed with the vector pOTE1 were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were 40 synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. Each value represents the mean (n=3)±standard deviation.

The substrate specificity of OtELo2 (SEQ ID NO: 81) could be determined after expression and the feeding of different fatty acids (Tab. 9). The substrates fed could be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the OtElo2 reaction. This means that the gene OtElo2 has been expressed functionally.

TABLE	9
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Fatty acid substrate	Conversion rate (in %)	
16:0	_	55
16:1 ^{Δ9}	_	55
16:3 ^{47,10,13}		
18:0	_	
$18:1^{\Delta 0}$		
$18:1^{\Delta 9}$		
$18:1^{\Delta 11}$	—	60
$18:2^{\Delta 9,12}$	—	00
$18:3^{\Delta 6,9,12}$	15.3±	
18:3 ^{45,9,12}	—	
$18:4^{\Delta 6,9,12,15}$	21.1±	
$20:2^{\Delta 11,14}$	—	
$20:3^{\Delta 8,11,14}$		
$20:4^{\Delta 5,8,11,14}$	—	65
$20:5^{\Delta 5,8,11,14,17}$	—	

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TABLE 9-continued

Conversion rate (in %)				
_				
_				

Table 9 shows the substrate specificity of the elongase OtElo2 for various fatty acids.

The yeasts which had been transformed with the vector pOTE2 were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. Each value represents the mean (n=3)±standard deviation.

The enzymatic activity shown in Table 9 clearly demonstrates that OTELO2 is a $\Delta 6$ -elongase.

Example 19

Cloning Genes from Thalassiosira pseudonana

The search for conserved regions in the protein sequences with the aid of the elongase genes with $\Delta 5$ -elongase activity $_{5}$ or $\Delta 6$ -elongase activity which are shown in the application allowed the identification of two sequences with suitable motifs in a Thalassiosira pseudonana sequence database (genomic sequences). The sequences were the following:

Name of gene	SEQ ID	Amino acids
TpELO1 (Δ5-elongase)	43	358
TpELO2 (Δ5-elongase)	59	358
TpELO3 (Δ6-elongase)	45	272

A 21 culture of T. pseudonana was grown in f/2 medium (Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. In Culture of Marine Invertebrate Animals (Eds. Smith, W. L. and Chanley, M. H.), Plenum Press, New York, pp 29-60) for 14 d (=days) at a light intensity of 80 E/cm². After the cells had been spun down, RNA was isolated with the aid of the RNAeasy Kit from Quiagen (Valencia, Calif., US) following the manufacturer's instructions. The mRNA was subjected to reverse transcription using the Marathon cDNA Amplification Kit (BD Biosciences) and adaptors were ligated in accordance with the manufacturer's instructions. Then, the cDNA library was used for the PCR for cloning expression plasmids by means of 5'- and 3'-RACE (rapid amplification of 50 cDNA ends).

Example 20

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

The relevant primer pairs were selected in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start 60 codon. The amplification of the TpElo DNAs was carried out in each case using 1 µl of cDNA, 200 µm of dNTPs, 2.5 U of Advantage polymerase and 100 pmol of each primer in a total volume of 50 µl. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 65 cycles of 30 seconds at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a last elongation step of 10 minutes at 72° C.

To clone the sequence for the heterologous expression in yeasts, the following oligonucleotides were used for the PCR reaction:

Name of gene and SEQ ID NO: Primer sequence						
	F:5'-accatgtgctcaccaccgccgtc (SEQ ID NO: 158) R:5'-ctacatggcaccagtaac (SEQ ID NO: 159)					
	F:5'-accatgtgctcatcaccgccgtc (SEQ ID NO: 160) R:5'-ctacatggcaccagtaac (SEQ ID NO: 161)					
	F:5'-accatggacgcctacaacgctgc (SEQ ID NO: 162) R:5'-ctaagcactcttctttt (SEQ ID NO: 163)					

*F = forward primer, R = reverse primer

The PCR products were incubated for 30 minutes at 21° C. with the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. The PCR product is ligated into the vector by means of a T overhang 25 and activity of a topoisomerase (Invitrogen). After incubation, E. coli DH5a cells were transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by means of Qiagen DNAeasy Kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector pYES2.1 was transformed in parallel. The yeasts were subsequently plated onto complete uracil dropout minimal medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1, pYES2.1-TpELO1, pYES2.1-TpELO2 and pYES2.1-TpELO3. After the selection, in each case two transformants were selected for further 40 functional expression.

Example 21

Cloning Expression Plasmids for the Seed Specific Expression in Plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3' termini of the coding sequences, using the following primer pair:

PSUN-TPELO1 Forward:	
(SEQ ID NO: 15	52)
Reverse: (SEQ ID NO: 15	53)
3 ' -GCGGCCGCCTACATGGCACCAGTAAC	
PSUN-TPELO2 Forward:	
(SEQ ID NO: 15	54)
Reverse: (SEQ ID NO: 15	55)
3 ' - GCGGCCGCCTACATGGCACCAGTAAC	

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-continued

PSUN-TPELO3 Forward:

(SEQ ID NO: 156) 5'-GCGGCCGCaccatqqacqcctacaacqctqc

------acggacycclacaddy

Reverse:

(SEQ ID NO: 157) 3'-GCGGCCGCCTAAGCACTCTTCTTCTTT

10 Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

¹⁵ 0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. PCR Reaction Conditions:

Annealing temperature: 1 min 55° C.

Denaturation temperature: $1 \min 94^\circ$ C.

Elongation temperature: 2 min 72° C.

Number of cycles: 35

The PCR products are incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP is incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector are separated by agarose gel electrophoresis and the corresponding DNA fragments are excised. The DNA is purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products are ligated. The Rapid Ligation Kit from Roche is used for this purpose. The resulting plasmids pSUN-TPELO1, pSUN-TPELO2 and pSUN-TPELO3 are verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene).

(Primer sequence:

SEQ ID NO: 151 5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCCGGATC

TGCTGGCTATGAA-3'));.

The PCR fragment was recut with EcoRI/Sall and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of *Arabidopsis* 65 *thaliana*, oilseed rape, tobacco and linseed.

Lipids were extracted from yeasts and seeds as described for Example 6.

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Example 22

Expression of TpELO1, TpELO2 and TpELO3 in Yeasts

Yeasts which had been transformed with the plasmids pYES2, pYES2-TpELO1, pYES2-TpELO2 and pYES2-TpELO3 as in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 in order to remove residual medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) dimethoxypropane. The FAMEs were extracted by twice extracting with petroleum ether (PE). To remove 20 nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0, and 2 ml of distilled water. Thereafter, the PE phases were dried with Na₂SO₄, evaporated under argon and taken up in 25 100 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. with an increment of 5° C./min and finally 10 minutes at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The 35 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- 40 218.

Example 23

Functional Characterization of TpELO1 and TpELO3

The substrate specificity of TpELO1 could be determined after expression and the feeding of different fatty acids (FIG. 9). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the TpEIo1 reaction. This means that the gene TpEIo1 has been expressed functionally.

It can be seen from Table 10 that TpEIo1 shows a narrow substrate specificity. TpEIo1 was only capable of elongating the C_{20} -fatty acids eicosapentaenoic acid and arachidonic acid, but preferred the ω 3-desaturated eicosapentaenoic ₆₀ acid.

The yeasts which had been transformed with the vector pYES2-TpELO1 were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

Expression of TpELO1 in yeast. Columns 1 and 3 show the control
reactions for columns 2 (fed: 250 µm 20:4 Δ5, 8, 11, 14)
and 4 (fed: 250 μ m 20:4 Δ 5, 8, 11, 14, 17).

Fatty acids	Expression 1	Expression 2	Expression 3	Expression 4
16:0	18.8	17.8	25.4	25.2
16:1 ^{Δ9}	28.0	29.8	36.6	36.6
18:0	5.2	5.0	6.8	6.9
18:1 ^{Δ9}	25.5	23.6	24.6	23.9
$20:4^{\Delta 5,8,11,14}$	22.5	23.4		
$22:4^{\Delta7,10,13,16}$		0.4		
$20:5^{\Delta 5,8,11,14,17}$			6.6	6.5
22:5 ^{\$\Delta7,10,13,16,19}				0.9
% conversion	0	1.7	0	12.2

The substrate specificity of TpElo3 could be determined after expression and the feeding of different fatty acids (FIG. **10**). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the TpElo3 reaction. This means that the gene TpElo3 has been expressed functionally.

It can be seen from Table 11 that TpElo3 shows a narrow substrate specificity. TpElo3 was only capable of elongating the C18-fatty acid γ -linolenic acid and stearidonic acid, but preferred the ω 3-desaturated stearidonic acid.

The yeasts which had been transformed with the vector pYES2-TpELO3 were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

TABLE 11

40	Expression of TpELO3 in yeast. Column 1 shows the fatty acid profile of yeast without feeding. Column 2 shows the control reaction. In columns 3 to 6, the following were fed: γ-linolenic acid, stearidonic acid, arachidonic acid and eicosapentaenoic acid (250 µm of each fatty acid).								
	Fatty acids	1	2	3	4	5	6		
	16:0 16:1 ^{Δ9}	17.9 41.7	20.6 18.7	17.8 27.0	16.7 33.2	18.8 24.0	18.8 31.3		
45	18:0 18:1 ^{Δ9}	7.0 33.3	7.7 16.8	6.4 24.2	6.6 31.8	5.2 25.5	6.0 26.4		
	$18:2^{\Delta9,12}$		36.1						
	18:3 ^{∆6,9,12} 18:4 ^{∆6,9,12,15}			6.1	1.7				
	$20:2^{\Delta 11,14}$	_	0	_	1./	_			
50	$20:3^{\Delta 8,11,14}$			18.5					
00	$20:4^{\Delta 8,11,14,17}$			_	10.0				
	$20:4^{\Delta 5,8,11,14}$	—	—	—	—	22.5			
	$22:4^{\Delta7,10,13,16}$	—	—	—	_	0			
	20:5 ^{45,8,11,14,17}	_	_	_	_	_	17.4		
	22:5 ^{\$\Delta7,10,13,16,19}						0		
55	% conversion	0	0	75	85	0	0		

Example 24

Cloning and Expression Plasmid for the Heterologous Expression of the Pi-omega3Des in Yeasts

For the heterologous expression in yeasts, the 65 Pi-omega3Des clone was cloned into the yeast expression vector pYES3 via PCR, using suitable Pi-omega3Des-specific primers. Here, exclusively the open reading frame, of

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the gene, which encodes the Pi-omega3Des protein was amplified and provided with two cleavage sites for cloning into the pYES3 expression vector:

(SEO ID NO: 149) Forward Primer: 5'-TAAGCTTACATGGCGACGAAGGAGG

(SEO ID NO: 150) Reverse Primer: 5'-TGGATCCACTTACGTGGACTTGGT

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

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

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl of the 5'ATG primer and 15 the 3' Stopp primer)

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. PCR Reaction Conditions:

Annealing temperature: 1 min 55° C.

Denaturation temperature: 1 min 94° C.

Elongation temperature: 2 min 72° C.

Number of cycles: 35

The PCR product was incubated with the restriction 25 enzymes HindIII and BamHI for 2 hours at 37° C. The yeast expression vector pYES3 (Invitrogen) was incubated in the same manner. Thereafter, the 1104 bp PCR product and the 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 desaturase cDNA were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pYES3-Pi-omega3Des was verified by sequencing and 35 transformed into the Saccharomyces strain INVSc1 (Invitrogen) by means of electroporation (1500 V). pYES3 was transformed in parallel to act as a control. Thereafter, the yeasts were plated onto complete minimal dropout tryptophan medium supplemented with 2% glucose. Cells which $_{40}$ were capable of growing in the medium without tryptophan thus comprise the relevant plasmids pYES3, pYES3-Piomega3Des. Following selection, in each case two transformants were selected for the further functional expression.

Example 25

Cloning Expression Plasmids for the Seed Specific **Expression** in Plants

A further transformation vector based on pSUN-USP was generated for the transformation of plants. To this end, NotI cleavage sites were introduced at the 5' and 3' termini of the coding sequence, using the following primer pair

PSUN-Pi-omega3Des

(SEQ ID NO: 149) Reverse: 3'-GCGGCCGCTTACGTGGACTTGGTC

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

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

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. 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 products were incubated with the restriction enzyme NotI for 4 hours at 37° C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp 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 products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pSUN-Piomega3Des was verified by sequencing.

Example 26

Expression of Pi-omega3Des in Yeasts

Yeasts which had been transformed with the plasmid pYES3 or pYES3-Pi-omega3Des, as described in Example 24, were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 in order to remove residual medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) dimethoxypropane. The FAMEs were extracted by twice extracting with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0, and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4 , evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50° C. to 250° C. with an increment of 5° C./min and finally 10 45 minutes at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding 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.

Example 23

Functional Characterization of Pi-omega3Des

The substrate specificity of Pi-omega3Des could be deter-60 mined after expression and the feeding of different fatty acids (FIGS. 12 to 18). The substrates fed are present in large amounts in all of the transgenic yeasts, which proves that these fatty acids have been taken up into the yeasts. The transgenic yeasts demonstrate the synthesis of novel fatty acids, the products of the Pi-omega3Des reaction. This means that the gene Pi-omega3Des has been expressed functionally.

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FIG. 12 represents the desaturation of linoleic acid (18:2 ω 6-fatty acid) to give α -linolenic acid (18:3 ω 3-fatty acid) by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (FIG. 12 A) or the 5 vector pYES3-Pi-omega3Des (FIG. 12 B) to acid methanolysis. The yeasts were cultured in minimal medium in the presence of $18:2^{\Delta9,12}$ -fatty acid (300 µm). Thereafter, the FAMEs were analyzed via GLC.

FIG. 13 represents the desaturation of γ -linolenic acid 10 (18:3 w6-fatty acid) to give stearidonic acid (18:4 w3-fatty acid) by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (FIG. 13 A) or the vector pYes3-Pi-omega3Des (FIG. 13 B) to acid methanoly- 15 sequences). The sequences were the following: sis. The yeasts were cultured in minimal medium in the presence of $\gamma C18:3^{\Delta6,9,12}$ -fatty acid (300 µm). Thereafter, the FAMEs were analyzed via GLC.

FIG. 14 represents the desaturation of C20:2- ω 6-fatty acid to give C20:3-w3-fatty acid by Pi-omega3Des. The 20 fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (FIG. 14 A) or the vector pYes3-Piomega3Des (FIG. 14 B) to acid methanolysis. The yeasts were cultured in miminal medium in the presence of C20: 25 $2^{\Delta11,14}\mbox{-fatty}$ acid (300 $\mu\mbox{m}).$ Thereafter, the FAMEs were analyzed via GLC.

FIG. 15 represents the desaturation of C20:3-w6-fatty acid to give C20:4-w3-fatty acid by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting 30 intact cells which had been transformed with the blank vector pYES2 (FIG. 15 A) or the vector pYes3-Piomega3Des (FIG. 15 B) to acid methanolysis. The yeasts were cultured in miminal medium in the presence of C20: $3^{\Delta 8,11,14}\text{-}\text{fatty}$ acid (300 µm). Thereafter, the FAMEs were ~35analyzed via GLC.

FIG. 16 shows the desaturation of arachidonic acid (C20: 4-(A)-6-fatty acid) to give eicosapentaenoic acid (C20:5ω3-fatty acid) by Pi-omega3Des.

The fatty acid methyl esters were synthesized by subject- 40 ing intact cells which had been transformed with the blank vector pYES2 (FIG. 16 A) or the vector pYes3-Piomega3Des (FIG. 16 B) to acid methanolysis. The yeasts were cultured in minimal medium in the presence of C20: $4^{\Delta5,8,11,14}$ -fatty acid (300 µm). Thereafter, the FAMEs were 45 analyzed via GLC.

FIG. 17 represents the desaturation of docosatetraenoic acid (C22:4-w6-fatty acid) to give docosapentaenoic acid (C22:5-w3-fatty acid) by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells 50 which had been transformed with the blank vector pYES2 (FIG. 17 A) or the vector pYes3-Pi-omega3Des (FIG. 17 B) to acid methanolysis. The yeasts were cultured in minimal medium in the presence of C22:4^{47,10,13,16}-fatty acid (300 μm). Thereafter, the FAMEs were analyzed via GLC. 55

The substrate specificity of Pi-omega3Des with regard to different fatty acids can be seen from FIG. 18. The yeasts which had been transformed with the vector pYes3-Piomega3Des were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters 60 were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. Each value represents a mean of three measurements. The conversion rates (% desaturation) were calculated using the formula:

[product]/[product]+[substrate]*100.

As described in Example 9, Pi-omega3Des can also be used for generating transgenic plants. Then, the lipids can be extracted from the seeds of these plants as described under Example 6.

Example 28

Cloning Desaturase Genes from Ostreococcus tauri

The search for conserved regions in the protein sequences with the aid of conserved motifs (H is boxes, Domergue et al. 2002, Eur. J. Biochem. 269; 4105-4113) allowed the identification of five sequences with corresponding motifs in an Ostreococcus tauri sequence database (genomic

Name of gei	ne SEQ ID	Amino acids	Homology
OtD4	SEQ ID NO: 95	536	Δ 4-desaturase
OtD5.1	SEQ ID NO: 91	201	Δ 5-desaturase
OtD5.2	SEQ ID NO: 93	237	Δ 5-desaturase
OtD6.1	SEQ ID NO: 89	456	Δ 6-desaturase
OtFad2	SEQ ID NO: 107	361	Δ 12-desaturase

The alignments for finding homologies of the individual genes were carried out using the tBLASTn algorithm (Altschul et al., J. Mol. Biol. 1990, 215:403-410).

The cloning procedure was as follows:

40 ml of an Ostreococcus tauri culture in the stationary phase were spun down, resuspended in 100 µl of doubledistilled water and stored at -20° C. The respective genomic DNAs were amplified on the basis of the PCR process. The relevant primer pairs were selected in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the OtDes DNAs was carried out in each case using 1 µl of defrosted cells, 200 µm of dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 µl. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 cycles of 30 seconds at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a last elongation step of 10 minutes at 72° C.

The following primers were employed in the PCR:

OtDes6.1 Forward:	
	(SEQ ID NO: 145)
5'ggtaccacataatgtgcgtggagacg	gaaaataacg3'
OtDes6.1 Reverse:	
	(SEQ ID NO: 146)
5'ctcgagttacgccgtctttccggagt	gttggcc3′

Example 29

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

To characterize the function of the desaturase OtDes6.1 = $\Delta 6$ -desaturase) from Ostreococcus tauri, the open reading frame of the DNA was cloned downstream of the galactoseinducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to the corresponding clone pYES2.1-OtDes6.1. Further desaturase genes from Ostreococcus can be cloned analogously.

The Saccharomyces cerevisiae strain 334 was transformed by electroporation (1500 v) with the vector pYES2.1-OtDes6.1. A yeast which was transformed with the blank vector pYES2 was used as the control. The transformed yeasts were selected on complete minimal dropout uracil medium (CMdum) agar plates supplemented with 2% glucose. After the selection, in each case three transformants ⁵ were selected for the further functional expression.

To express the OtDes6.1 desaturase, precultures of in each case 5 ml of dropout uracil CMdum liquid medium supplemented with 2% (w/v) raffinose were inoculated with the selected transformants and incubated for 2 days at 30° C., 10 200 rpm. 5 ml of CMdum liquid medium (without uracil) supplemented with 2% raffinose and $300 \,\mu\text{m}$ of various fatty acids were then inoculated with the precultures to an OD₆₀₀ of 0.05. Expression was induced by addition of 2% (w/v) galactose. The cultures were incubated for a further 96 hours 15 at 20° C.

Example 30

Cloning of Expression Plasmids for the Seed-Specific Expression in Plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3' termini of the 25 coding sequences, using PCR. The corresponding primer sequences are derived from the 5' and 3' regions of the desaturases.

Composition of the PCR Mix (50 μ l):

5.00 µl template cDNA

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

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. 35 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 products were incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the vector were separated by agarose gel electrophoresis and the corresponding DNA 45 fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids were verified by 50 sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated 55 from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Ostreococcus gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, 60 M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession 65 X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment

which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene). (Primer sequence: 5'-GTCGAC-CCGCGGACTAGTGGGCCCTCTAGAC-

CCGGGGGGATCC GGATCTGCTGGCTATGAA-3', SEQ ID NO: 144).

The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of *Arabidopsis thaliana*, oilseed rape, tobacco and linseed.

Example 31

Expression of OtDes6.1 in Yeasts

Yeasts which had been transformed with the plasmids pYES2, pYES2-OtDes6.2 as described in Example 4 were ²⁰ analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80° C. together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4 , evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50° C. to 250° C. with an increment of 5° C./min and finally 10 min at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding 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.

Example 32

Functional Characterization of Desaturases from Ostreococcus

The substrate specificity of desaturases can be determined after expression in yeast (see examples Cloning desaturase genes, Yeast expression) by feeding by means of different yeasts. Descriptions for determining the individual activities are found in WO 93/11245 for Δ 15-desaturases, WO 94/11516 for Δ 12-desaturases, WO 93/06712, U.S. Pat. No. 5,614,393, U.S. Pat. No. 5,614,393, WO 96/21022, WO 0021557 and WO 99/27111 for Δ 6-desaturases, Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566 for Δ 4-desaturases, Hong et al. 2002, Lipids 37, 863-868 for Δ 5-desaturases.

Table 12 represents the substrate specificity of the desaturase OtDes6.1 with regard to different fatty acids. The substrate specificity of OtDes6.1 was determined after

expression and feeding of various fatty acids. The substrates which have been fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the OtDes6.2 reaction (FIG. **20**). This means that the gene ⁵ OtDes6.1 has been expressed functionally.

The yeasts which had been transformed with the vector pYES2-OtDes6.1 were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. Each value represents the mean $(n=3)\pm$ standard deviation. The activity corresponds to the conversion rate calculated using the formula [substrate/(substrate+product)*100].

It can be seen from Table 12 that OtDes6.1 shows ¹⁵ substrate specificity for linoleic and linolenic acid (18:2 and 18:3) since the highest activities are obtained with these

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together with the *Physcomitrella patens* Δ 6-elongase PSE1 (Zank et al. 2002, Plant J. 31:255-268) and the *Phaeodac-tylum tricornutum* Δ 5-desaturase PtD5 (Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113) to give dihomo- γ -linolenic acid (=DHGLA) and arachidonic acid (=ARA, FIG. **21**B) and dihomostearidonic acid (=DHSTA) and eicosapentaenoic acid (=EPA, FIG. **21**D), respectively. FIG. **21** shows clearly that the reaction products GLA and STA of the Δ 6-desaturase OtDes6.1 in the presence of the Δ 6-elongase PSE1 is elongated virtually quantitatively to give DHGLA and DHSTA, respectively. The subsequent desaturation by the Δ 5-desaturase PtD5 to give ARA and EPA, respectively, also proceeds smoothly. Approximately 25-30% of the elongase product is desaturated (FIGS. **21**B and D).

TABLE 13

which follows gives an overview of the Ostreococcus desaturases which have been cloned: Ostreococcus tauri desaturases							
Name	bp	aa Homology	Cyt. B5	His box1	His box2	His box3	
OtD4	1611	536∆4-desaturase		(SEQ ID	WRYHHQVSH (SEQ ID NO: 231)	(SEQ ID	
OtD5.1	606	201∆5-desaturase	-	-	_	QVVHHLFP (SEQ ID NO: 236)	
OtD5.2	714	237∆5-desaturase	-	-	WRYHHMVSHI (SEQ ID NO: 232)	(SEQ ID	
OtD6.1	1443	480∆6-desaturase	· ~	(SEQ ID	WNSMHNKHH (SEQ ID NO: 233)	-	
Qt FAD2	1086	361∆12-desaturase	-	HECGH (SEQ ID NO: 230)	WQRSHAVHH (SEQ ID NO: 234)	(SEQ ID	

fatty acids. In contrast, the activity for oleic acid (18:1) and palmitoleic acid (16:1) is markedly lower. The preferred $_{45}$ conversion of linoleic and linolenic acid demonstrates that this desaturase is suitable for the production of polyunsaturated fatty acids.

Substrates	Activity in %	50
 16:1 ^{Δ9}	5.6	
$18:1^{\Delta 9}$	13.1	
$18:2^{\Delta 9,12}$	68.7	
$18:3^{\Delta 9,12,15}$	64.6	55

FIG. **20** shows the conversion of linoleic acid by OtDes6.1. The FAMEs were analyzed via gas chromatography. The substrate which has been fed (C18:2) is converted into γ -C18:3. Both the starting material and the 60 resulting product are indicated by arrows.

FIG. **21** represents the conversion of linoleic acid (=LA) and α -linolenic acid (=ALA) in the presence of OtDes6.1 to give γ -linolenic acid (=GLA) and stearidonic acid (=STA), respectively (FIGS. **21**A and C). Moreover, FIG. **21** shows 65 the conversion of linoleic acid (=LA) and α -linolenic acid (=ALA) in the presence of the Δ 6-desaturase OtDes6.1

Example 33

Cloning Desaturase Genes from Thalassiosira pseudonana

The search for conserved regions in the protein sequences 50 with the aid of conserved motifs (His boxes, see motifs) allowed the identification of six sequences with corresponding motifs in an *Thalassiosira pseudonana* sequence database (genomic sequences). The sequences were the following:

	Name of gene	SEQ ID	Amino acids	Homology
C	TpD4	SEQ ID NO: 103	503	Δ 4-desaturase
	TpD5-1	SEQ ID NO: 99	476	Δ 5-desaturase
	TpD5-2	SEQ ID NO: 101	482	Δ 5-desaturase
	TpD6	SEQ ID NO: 97	484	Δ 6-desaturase
	TpFAD2	SEQ ID NO: 109	434	Δ 12-desaturase
	TpO3	SEQ ID NO: 105	418	ω 3-desaturase

The cloning procedure was as follows:

40 ml of an *Thalassiosira pseudonana* culture in the stationary phase were spun down, resuspended in 100 of

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double-distilled water and stored at -20° C. The respective genomic DNAs were amplified on the basis of the PCR method. The relevant primer pairs were selected in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, *Cell* 1986, 44:283-292) next to the start codon. The amplification of the TpDes DNAs was carried but in each case using 1 µl of defrosted cells, 200 µm of dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 µl. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 cycles of 30 seconds at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a last elongation step of 10 minutes at 72° C.

Example 34

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

To characterize the function of the desaturases from *Thalassiosira pseudonana*, the open reading frame of the respective DNA was cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to the corresponding pYES2.1 clone. 25

The *Saccharomyces cerevisiae* strain 334 is transformed by electroporation (1500 v) with the vectors pYES2.1-TpDesaturasen. A yeast which is transformed with the blank vector pYES2 is used as the control. The transformed yeasts are selected on complete minimal dropout uracil medium ³⁰ (CMdum) agar plates supplemented with 2% glucose. After the selection, in each case three transformants are selected for the further functional expression.

To express the Tp desaturases, initially precultures of in each case 5 ml of dropout uracil CMdum liquid medium ³⁵ supplemented with 2% (w/v) raffinose are inoculated with the selected transformants and incubated for 2 days at 30° C., 200 rpm. 5 ml of liquid CMdum medium (without uracil) supplemented with 2% raffinose and 300 µm of various fatty acids are then inoculated with the precultures to an OD₆₀₀ of ⁴⁰ 0.05. The expression is induced by addition of 2% (w/v) galactose. The cultures are incubated for a further 96 hours at 20° C.

Example 35

Cloning of Expression Plasmids for the Seed-Specific Expression in Plants

A further transformation vector based on pSUN-USP is 50 generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3" termini of the coding sequences, using PCR. The corresponding primer sequences are derived from the 5' and 3' regions of the desaturases. 55

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

 $5.00 \ \mu l \ 10 \times buffer \ (Advantage \ polymerase)+25 \ mM \ MgCl_2$

- 5.00 µl 2 mM dNTP
- 1.25 µl of each primer (10 pmol/µl)
- 0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. PCR reaction conditions:

- Annealing temperature: 1 min 55° C.
- Denaturation temperature: 1 min 94° C.
- Elongation temperature: $2 \min 72^\circ$ C.

Number of cycles: 35

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The PCR products are incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP is incubated in the same manner. Thereafter, the PCR products and the vector are separated by agarose gel electrophoresis and the corresponding DNA fragments are excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products are ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids are verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is the OCS gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene).

(Primer sequence:

SEQ ID NO: 143 GTCGACCCGCGGGACTAGTGGGCCCTCTAGACCCGGGGGATCCGGATCTGC

TGGCTATGAA3',).

The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of *Arabidopsis thaliana*, oilseed rape, tobacco and linseed.

Example 36

Expression of Tp Desaturases in Yeasts

Yeasts which have been transformed with the plasmids pYES2 and pYES2-TpDesaturasen as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures are harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) are prepared by acid methanolysis. To this end, the cell sediments are incubated for one hour at 80° C. together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases are washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases are dried with Na₂SO₄, evaporated under argon and taken up in 100 µl of PE. The samples are separated on a DB-23 capillary column (30 m,

0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis are as follows: the oven temperature is programmed from 50° C. to 250° C. with an $_{5}$ increment of 5° C./min and finally 10 min at 250° C. (holding).

The signals are identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and 10 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.

Example 37

Functional Characterization of Desaturases from Thalassiosira pseudonana

The substrate specificity of desaturases can be determined after expression in yeast (see examples Cloning desaturase genes, Yeast expression) by feeding by means of different yeasts. Descriptions for determining the individual activities 25 are found in WO 93/11245 for $\Delta 15$ -desaturases. WO 94/11516 for Δ12-desaturases, WO 93/06712, U.S. Pat. No. 5,614,393, U.S. Pat. No. 5,614,393, WO 96/21022, WO 0021557 and WO 99/27111 for $\Delta 6$ -desaturases, Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566 for Δ4-desaturases, ³⁰ Hong et al. 2002, Lipids 37, 863-868 for Δ 5-desaturases.

The activity of the individual desaturases is calculated from the conversion rate using the formula [substrate/(substrate+product)*100]

Tables 11 and 12 which follow give an overview of the 35 cloned Thalassiosira pseudonana desaturases.

TABLE 14

Length and characteristic features of the cloned Thalassiosira pseudonana desaturases						
Desaturase	cDNA H (bp)	Proteir (aa)		His box1	lHis box2	His box3
TpD4	1512	503		(SEQ ID	WELQHMLGHH (SEQ ID NO: 244)	QIEHHLFP (SEQ ID NO: 250)
TpD5-1	1431	476		HDANH (SEQ ID NO: 241)	WMAQHWTHH (SEQ ID NO: 245)	QVEHHLFP (SEQ ID NO: 235)
TpD5-2	1443	482		(SEQ ID	WLAQHWTHH (SEQ ID NO: 246)	QVEHHLFP (SEQ ID NO: 235)
TpD6	1449	484		· ~	WKNKHNGHH (SEQ ID NO: 247)	QVDHHLFP (SEQ ID NO: 251)
TpFAD2 (d12)	1305	434	-		HAKHH (SEQ ID NO: 248)	HVAHHLFH (SEQ ID NO: 252)
ТрОЗ	1257	419	-		WLFMVTYLQHH (SEQ ID NO: 249)	HWHHLF (SEQ ID NO: 253)

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INBEL 15							
Length, axons, homology and identities of the cloned desaturases.							
Des.	GDN A (bp) Exon 1	Exon 2	First Blast Hit	Hom./ Iden.			
TpD4	2633 496-131	4 1571-2260	Thrautochitrium	56%/43%			
TpD5-1	2630 490-800	900-2019	D4-des <i>Phaeodactylum</i> D5-des	74%/62%			
TpD5-2	2643 532-765	854-2068	Phaeodactylum D5-des	72%/61%			
TpD6	2371 379-480	630-1982	Phaeodactylum D6-des	83%/69%			
TpFAD2	2667 728-203	2 —	Phaeodacrylum FAD2	76%/61%			
ТрО3	2402 403-988	1073-1743	FAD2 Chaenorhabdidis Fad2	49%/28%			

The $\Delta 12$ -desaturase genes from Ostreococcus and Thal-20 assiosira can also be cloned analogously to the above examples.

Example 38

Cloning Elongase Genes from Xenopus laevis and Ciona intestinalis

The search for conserved regions (see consensus sequences, SEQ ID NO: 115 and SEQ ID NO: 116) in the protein sequences in gene databases (Genbank) with the aid of the elongase genes with $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity, which are detailed in the application, allowed the identification and isolation of further elongase sequences from other organisms. Further sequences were identified in each case from X. laevis and from C. intestinalis, using suitable motifs. The sequences were the following:

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Name of gene	Organism	Genbank No.	SEQ ID NO:	Amino acids	
ELO(XI)	Xenopus laevis	BC044967	117	303	
ELO(Ci)	Ciona intestinalis	AK112719	119	290	

The cDNA clone of *X. laevis* was obtained from the NIH (National Institute of Health) [Genetic and genomic tools for *Xenopus* research: The NIH *Xenopus* initiative, Dev. Dyn. 225 (4), 384-391 (2002)].

The cDNA clone of *C. intestinalis* was obtained from the University of Kyoto [Satou, Y., Yamada, L, Mochizuki, Y., Takatori, N, Kawashima, T., Sasaki, A., Hamagu-chi, M., ¹⁵ Awazu, S., Yagi, K., Sasakura, Y., Nakayama, A., Ishikawa, H., Inaba, K. and Satoh, N. "A cDNA resource from the basal chordate Ciona intestinalis" JOURNAL Genesis 33 (4), 153-154 (2002)].

Example 39

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

The elongase DNAs were amplified in each case using 1 μ l of cDNA, 200 μ M dNTPs, 2.5 U of Advantage polymerase and 100 pmol of each primer in a total volume of 50 μ l. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 cycles of 30 seconds 30 at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a final elongation step of 10 minutes at 72° C.

To clone the sequence for heterologous expression in yeasts, the following oligonucleotides were used for the PCR reaction:

Name of gene and SEQ ID NO	: Primer sequence	
-	1 F:5'-AGGATCC <u>ATG</u> GCCTTCAAGGAGCTCACATC 2 R:5'-CCTCGAG <u>TCA</u> ATGGTTTTTGCTTTTCAATGC ACCG	
	3 F:5'-TAAGCTT <u>ATG</u> GACGTACTTCATCGT 4 R:5'-TCAGATCT <u>TTA</u> ATCGGTTTTACCATT	

*F = forward primer, R = reverse primer

The PCR products were incubated for 30 minutes at 21° C. with the yeast expression vector pYES2.1-TOPO (Invit- 50 rogen) following the manufacturer's instructions. The PCR product is ligated into the vector by means of a T overhang and activity of a topoisomerase (Invitrogen). After incubation, E. coli DH5 α cells were transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by 55 means of Qiagen DNAeasy Kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector pYES2.1 was transformed in parallel. The yeasts were subsequently plated 60 onto complete uracil dropout minimal medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1, pYES2.1-ELO(XI) and pYES2.1-ELO(Ci). After the selection, in each case two 65 transformants were selected for further functional expression.

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Example 40

Cloning Expression Plasmids for the Seed-Specific Expression in Plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:

pSUN-ELO(XI) Forward:				
5 ' - GCGGCCGCACCATGGCCTTCAAGGAGC	· ~		NO :	125)
Reverse:	(900	тп	NO.	126)
3 ' - GCGGCCGCCTTCAATGGTTTTTGCTTT				120)
pSUN-ELO(Ci) Forward:	(CEO	TD	NO	127)
5 ' - GCGGCCGCACC <u>ATG</u> GACGTACTTCATC	. ~	10	110:	127)
Reverse:	(550	тп	NO	128)
3 ' - GCGGCCGCTTTAATCGGTTTTACCATT	(PEQ	тр	110.	1207

Composition of the PCR Mix $(50 \ \mu l)$:

5.00 µl template cDNA

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

5.00 μl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. 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 products were incubated with the restriction enzyme NotI for 16 hours at . . . 37° C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp 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 products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids pSUN-ELO(XI) and pSUN-ELO(Ci) were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Octopine synthase gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR

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reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene).

Primer sequence:

(SEQ ID NO: 129) 5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCCGGATC

TGCTGGCTATGAA-3'.

The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of *Arabidopsis thaliana*, oilseed rape, tobacco and linseed.

Lipids were extracted from yeasts and seeds as described for Example 6.

Example 41

Expression of ELO(XI) and ELO(Ci) in Yeasts

Yeasts which had been transformed with the plasmids pYES2, pYES2-ELO(XI) and pYES2-ELO(Ci) as in Example 4 were analyzed as follows: 25

The yeast cells from the main cultures were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 in order to remove residual medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. with 2 ml of 1N methanolic sulfuric acid and 2% (v/v) dimethoxypropane. The FAMEs were extracted by twice extracting with petroleum ether (PE). To remove non-de- 35 rivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0, and 2 ml of distilled water. Thereafter, the PE phases were dried with Na₂SO₄, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary 40 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. with an increment of 5° C./min and finally 10 45 minutes at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids 36(8):761-766; Sayanova et al., ⁵⁰ 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.

Example 42

Functional Characterization of ELO(XI) and ELO(Ci)

The substrate specificity of ELO(XI) can be determined after expression and the feeding of different fatty acids (FIG. **22**). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the 65 ELO(XI) reaction. This means that the gene ELO(XI) has been expressed functionally.

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It can be seen from Table 16 that ELO(XI) shows a broad substrate specificity. Both C18- and C₂₀-fatty acids are elongated, but a preference for Δ 5- and Δ 6-desaturated fatty acids can be observed.

The yeasts which had been transformed with the vector pYES2-ELO(XI) were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

TABLE 16

_	Expression of ELO(XI) in yeast. The conversion rate of different starting materials (amounts fed: in each case 250 μ M) is described.			
	Starting materials	Conversion of the starting materials by ELO(XI) in %		
	16:0	3		
	16:1 ^{Δ9}	0		
	18:0	2		
	18:1 ⁴⁹	0		
	18:2 ^{49,12}	3		
	18:3 ^{46,9,12}	12		
	18:3 ^{45,9,12}	13		
	18:3 ^{49,12,15}	3		
	$18:4^{\Delta 6,9,12,15}$	20		
	20:3 ^{48,11,14}	5		
	$20:3^{\Delta 11,14,17}$	13		
	$20:4^{\Delta 5,8,11,14}$	15		
	$20:5^{\Delta 5,8,11,14,17}$	10		
	$22:4^{\Delta7,10,13,16}$	0		
	22:6 ^{Δ4,7,10,13,16,19}	Ő		

The substrate specificity of ELO(Ci) can be determined after expression and the feeding of different fatty acids (FIG. **23**). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the ELO(Ci) reaction. This means that the gene ELO(Ci) has been expressed functionally.

TABLE 17

different starting materials (amounts fed: in		
each case 250 µM) is described.		
Starting materials	Conversion of the starting materials by ELO(Ci) in %	

Starting materials	ELO(Ci) in %	
16:0	0	
16:1 ^{Δ9}	0	
18:0	0	
18:1 ^{Δ9}	0	
18:2 ^{49,12}	23	
$18:3^{\Delta 6,9,12}$	10	
18:3 ^{45,9,12}	38	
18:3 ^{49,12,15}	25	
$18:4^{\Delta 6,9,12,15}$	3	
20:3 ^{48,11,14}	10	
$20:3^{\Delta 11,14,17}$	8	
$20:4^{\Delta 5,8,11,14}$	10	
$20:5^{\Delta 5,8,11,14,17}$	15	
$22:4^{\Delta7,10,13,16}$	0	
22:6 ^{Δ4,7,10,13,16,19}	0	

It can be seen from Table 17 that ELO(Ci) shows a broad substrate specificity. Both C18- and C20-fatty acids are elongated, but a preference for Δ 5- and Δ 6-desaturated fatty acids can be observed.

The yeasts which had been transformed with the vector pYES2-ELO(Ci) were cultured in minimal medium in the

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presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

Example 43

Cloning Genes from Ostreococcus tauri

The search for conserved regions in the protein sequences ¹⁰ with the aid of the elongase genes with $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity, which have been described herein, allowed the identification of in each case two sequences with corresponding motifs in an *Ostreococcus tauri* sequence ¹⁵ database (genomic sequences). The sequences were the ¹⁵ following:

Name of gene	SEQ ID	Amino acids
OtELO1, (Δ5-elongase)	SEQ ID NO: 67	300
OtELO1.2, (Δ 5-elongase)	SEQ ID NO: 113	300
OtELO2, (A6-elongase)	SEQ ID NO: 69	292
OtELO2.1, ($\Delta 6$ -elongase)	SEQ ID NO: 111	292

OtElo1 and OtElo1.2 show the highest similarity with an elongase from *Danio rerio* (GenBank AAN77156; approximately 26% identity), while OtElo2 and OtElo2.1 show the highest similarity with *Physcomitrella* Elo (PSE) [approx. 36% identity] (alignments were carried out using the 30 tBLASTn algorithm (Altschul et al., J. Mol. Biol. 1990, 215: 403-410)).

The elongases were cloned as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down, resuspended in 100 μ l of doubledistilled water and stored at -20° C. The respective genomic DNAs were amplified on the basis of the PCR method. The relevant primer pairs were selected in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start 40 codon. The amplification of the OtElo DNAs was carried out in each case using 1 μ l of defrosted cells, 200 μ M of dNTPs, 2.5 U Taq polymerase and 100 pmol of each primer in a total volume of 50 μ l. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 cycles 45 of 30 seconds at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a last elongation step of 10 minutes at 72° C.

Example 44

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

To characterize the function of the elongases from *Ostreococcus tauri*, the open reading frames of the respective 55 DNAs were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to pOTE1, pOTE1.2, pOTE2 and pOTE2.1.

The *Saccharomyces cerevisiae* strain 334 was transformed by electroporation (1500 V) with the vector pOTE1, 60 pOTE1.2, pOTE2 and pOTE2.1, respectively. A yeast which was transformed with the blank vector pYES2 was used as the control. The transformed yeasts were selected on complete minimal dropout uracil medium (CMdum) agar plates supplemented with 2% glucose. After the selection, in each 65 case three transformants were selected for the further functional expression.

To express the Ot elongases, precultures of in each case 5 ml of liquid CMdum medium supplemented with 2% (w/v) raffinose, but without uracil, were inoculated with the selected transformants and incubated for 2 days at 30° C., 200 rpm. 5 ml of liquid CMdum medium (without uracil) supplemented with 2% raffinose and 300 μ m of various fatty acids were then inoculated with the precultures to an OD₆₀₀ of 0.05. The expression was induced by addition of 2% (w/v) galactose. The cultures were incubated for a further 96 hours at 20° C.

Example 45

Cloning of Expression Plasmids for the Seed-Specific Expression in Plants

A further transformation vector based on pSUN-USP was generated for the transformation of plants. To this end, NotI ²⁰ cleavage sites were introduced at the 5' and 3' ends of the coding sequences, using PCR. The corresponding primer sequences were derived from the 5' and 3' regions of OtElo1, OtElo1.2, OtElo2 and OtElo2.1.

Composition of the PCR Mix (50 μ l):

5.00 µl template cDNA

5.00 μ l 10× buffer (Advantage polymerase)+25 mM MgCl₂ 5.00 μ l 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed. 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 products are incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP is incubated in the same manner. Thereafter, the PCR products and the 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 products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids pSUN-OtELO1, pSUN-OtELO1.2, pSUN-OtELO2 and pSUN-OtELO2.2 were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga; P., (-1994) The small versatile pPZP 50 family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Ostreococcus gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene).

Primer sequence: (SEQ ID NO: 130) 5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCCGGATC

TGCTGGCTATGAA-3', .

The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of *Arabidopsis* 10 *thaliana*, oilseed rape, tobacco and linseed.

Example 46

Expression of OtElo1, OtElo1.2, OtElo2 and OtELO2.2 in Yeasts

Yeasts which had been transformed with the plasmids pYES3, pYES3-OtEIO1, pYES3-OtEIO1.2, pYES3-OtELO2 and pYES3-OtELO2.2 as described in Example 15²⁰ were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 to remove residual medium and fatty 25 acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted $_{30}$ twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO3, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4 , evaporated under argon and taken up in 100 µl of 35 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 equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50° $\,^{40}$ C. to 250° C. with an increment of 5° C./min and finally 10 min at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and ⁴⁵ 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):215-50 218.

Example 47

Functional Characterization of OtElo1, OtElo1.2, OtElo2 and OtElo2.1

The substrate specificity of OtElo1 was determined after expression and feeding of different fatty acids (Table 18). The substrates which have been fed can be detected in large 6 amounts in all transgenic yeasts. The transgenic yeasts showed the synthesis of novel fatty acids, the products of the OtElo1 reaction. This means that the gene OtElo1 was expressed functionally.

It can be seen from Table 18 that OtElo1 and OtElo1.2 6 have a narrow substrate specificity. OtElo1 and OtElo1.2 were only capable of elongating the C20-fatty acids eicosapentaenoic acid (FIG. 24A, 24B) and arachidonic acid (FIG. 25A, 25B), but preference was given to the ω 3-desaturated eicosapentaenoic acid.

Table 18 shows the substrate specificity of the elongase OtElo1 and OtElo1.2 for C20-poly unsaturated fatty acids with a double bond in the Δ 5-position in comparison with different fatty acids.

The yeasts which had been transformed with the vector pOTE1 or pOTE1.2 were cultured in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

The substrate specificity of OtElo2 (SEQ ID NO: 81)
 OtElo2.1 (SEQ ID NO: 111) can be determined after expression and the feeding of different fatty acids (Table 19). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the OtElo2
 reaction; This means that the genes OtElo2 and OtElo2.1 have been expressed functionally.

TABLE 18

Fatty acid substrate	Conversion rate of OtElo1 (in %)	Conversion rate of OtElo1.2 (in %)
16:0	_	_
16:1 ^{Δ9}	_	_
18:0	_	_
18:1 ^{Δ9}	_	_
$18:1^{\Delta 11}$	—	_
$18:2^{\Delta 9,12}$	_	_
$18:3^{\Delta 6,9,12}$	_	_
18:3 ^{45,9,12}	_	_
$20:3^{\Delta 8,11,14}$	_	_
$20:4^{\Delta 5,8,11,14}$	10.8 ± 0.6	38.0
$20:5^{\Delta 5,8,11,14,17}$	46.8 ± 3.6	68.6
22:4 ^{47,10,13,16}	_	_
22:6 ^{4,7,10,13,16,19}	—	_

Table 19 shows the substrate specificity of the elongase OtElo2 and OtElo2.1 with regard to various fatty acids. OtElo2.1 shows a markedly higher activity.

The yeasts which had been transformed with the vector pOTE2 or pOTE2.1 were cultured in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

The enzymatic activity shown in Table 19 clearly demonstrates that OtElo2 and OtElo2.1, respectively, are a $\Delta 6$ -elongase.

TABLE 19

_			
5	Fatty acid substrate	Conversion rate of OtElo2 (in %)	Conversion rate of OtElo2.2 (in %)
	16:0	_	_
	$16:1^{\Delta 9}$	_	
	16:3 ^{47,10,13}	_	_
	18:0	_	_
. 18:1 ^{∆6}			_
0	$18:1^{\Delta 9}$	_	
	$18:1^{\Delta 11}$		_
	$18:2^{\Delta 9,12}$		_
	18:3 ^{46,9,12}	15.3	55.7
	18:3 ^{45,9,12}	_	_
5 $20:2^{\Delta 11,1}$	$18:4^{\Delta 6,9,12,15}$	21.1	70.4
	$20:2^{\Delta 11,14}$	_	_
	20:3 ^{Δ8,11,14}	_	_

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TABLE 19-continued				
Fatty acid substrate	Conversion rate of OtElo2 (in %)	Conversion rate of OtElo2.2 (in %)		
20:4 ^{45,8,11,14}	_	_		
$20:5^{\Delta 5,8,11,14,17}$	_			

22:4^{Δ7,10,13,16}

22:5^{\$\$\Delta7,10,13,16,19}

22:6^{Δ4,7,10,13,16,19}

FIG. 24 A-D shows the elongation of eicosapentaenoic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22: 5ω3).

FIG. 25 A-D shows the elongation of arachidonic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22:4 ω 6).

Example 48

Cloning Elongase Genes from Euglena gracilis and Arabidopsis thaliana

The search for conserved regions in the protein sequences with the aid of the elongase genes with $\Delta 5$ -elongase activity or $\Delta 6$ -elongase activity, which are detailed in the application, allowed the identification of sequences from Arabidopsis thaliana and Euglena gracilis, respectively, with 30 corresponding motifs in sequence databases (Genbank, Euglena EST Bank). The sequences were the following:

Name of gene	SEQ ID	Amino acids
EGY1019 (E. gracilis)	SEQ ID NO: 131	262
EGY2019 (E. gracilis)	SEQ ID NO: 133	262
At3g06460 (A. thaliana)	SEQ ID NO: 135	298
At3g06470 (A. thaliana)	SEQ ID NO: 137	278

The Euglena gracilis elongases were cloned as follows:

The Euglena gracilis strain 1224-5/25 was obtained from the Sammlung für Algenkulturen Göttingen [Göttingen collection of algal cultures] (SAG). For the isolation, the strain $_{45}$ was grown for 4 days at 23° C. in medium II (Calvayrac R and Douce R, FEBS Letters 7:259-262, 1970) with a photoperiod of 8 h/16 h (light intensity 35 mol s-1m-2).

Total RNA of a four-day-old Euglena culture was isolated with the aid of the RNAeasy Kit from Qiagen (Valencia, 50 Calif., US). poly-A+ RNA (mRNA) was isolated from the total RNA with the aid of oligo-dT-cellulose (Sambrook et al., 1989). The RNA was subjected to reverse transcription with the Reverse Transcription System Kit from Promega, and the cDNA synthesized was cloned into the lambda ZAP 55 vector (lambda ZAP Gold, Stratagene). The cDNA was depackaged in accordance with the manufacturer's instructions to give the plasmid DNA, and clones were partially sequenced for random sequencing. mRNA was isolated from the total RNA with the aid of the PolyATract isolation 60 system (Promega). The mRNA was subjected to reverse transcription with the Marathon cDNA Amplification Kit (BD Biosciences) and the adaptors were ligated in accordance with the manufacturer's instructions. The cDNA library was then used for the PCR for cloning expression 65 plasmids by means of 5'- and 3'-RACE (rapid amplification of cDNA ends).

The Arabidopsis thaliana elongases were cloned as follows:

Starting from the genomic DNA, primers for the two genes were derived at the 5' and the 3' end of the open reading frame.

The method of Chrigwin et al., (1979) was used for isolating total RNA from A. thaliana. Leaves from 21-dayold plants were crushed in liquid nitrogen, treated with disruption buffer and incubated for 15 minutes at 37° C. After centrifugation (10 min, 4° C., 12 000×g), the RNA in the supernatant was precipitated at -20° C. for 5 hours using 0.02 volume of 3 M sodium acetate pH 5.0 and 0.75 volume ethanol. After a further centrifugation step, the RNA was taken up in 1 ml of TES per g of starting material, extracted once with one volume of phenol/chloroform and: once with one volume of chloroform, and the RNA was precipitated with 2.5 M LiCl. Following subsequent centrifugation and washing with 80% ethanol, the RNA was resuspended in water. The cDNA was synthesized in accordance with the method of Sambrook et al. 1989, and an RT-PCR was carried 20 out using the derived primers. The PCR products were cloned into the vector pYES2.1-TOPO (Invitrogen) in accordance with the manufacturer's instructions.

Example 49

Cloning Expression Plasmids for Heterologous Expression in Yeasts

To characterize the function of the A. thaliana elongases, the open reading frames of the DNAs in question were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to pAt60 and pAt70.

The Saccharomyces cerevisiae strain 334 was transformed by electroporation (1500 V) with the vector pAt60 ³⁵ and pAt70, respectively. A yeast which was transformed with the blank vector pYES2.1 was used as the control. The transformed yeasts were selected on complete minimal dropout uracil medium (CMdum) agar plates supplemented with 2% glucose. After the selection, in each case three 40 transformants were selected for the further functional expression.

To express the At elongases, precultures of in each case 5 ml of dropout uracil CMdum liquid medium supplemented with 2% (w/v) raffinose were inoculated with the selected transformants and incubated for 2 days at 30° C., 200 rpm.

5 ml of liquid CMdum medium (without uracil) supplemented with 2% raffinose and 300 µM of various fatty acids were then inoculated with the precultures to an OD_{600} of 0.05. The expression was induced by addition of 2% (w/v) galactose. The cultures were incubated for a further 96 hours at 20° C.

Example 50

Expression of pAt60 and pAt70 in Yeasts

Yeasts which had been transformed with the plasmids pYES2.1, pAt60 and pAt70 as described in Example 5 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100×g, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by add methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted

twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO3, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4 , evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50° 10C. to 250° C. with an increment of 5° C./min and finally 10 min at 250° C. (holding).

The signals were identified by comparing the retention times with corresponding 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.

Example 51

Functional Characterization of pAt60 and pAt70

The substrate specificity of the elongases At3g06460 and At3g06470 was determined after expression and feeding of various fatty acids (Table 20, FIG. 26). The substrates which have been fed can be detected in all transgenic yeasts. The transgenic yeasts showed the synthesis of novel fatty acids, 30 the products of the genes At3g06460 and At3g06470, respectively. This means that these genes have been expressed functionally.

TABLE 20

Elongation of EPA by the elongases At3g06460 and At3g06470, respectively. Measurement of the yeast extracts after feeding of 250 μM EPA				
Gene	Fatty acid fed	C20:5n-3 content	C22:5n-3 content	4
At3g06460 At3g06460 Conversion ra	EPA (C20:5n-3) EPA (C20:5n-3) te of EPA	20.8 25.4 At3g06460: 3.0%	0.6 1.1 At3g06470: 4.1%	

FIG. 26 represents the elongation of 20:5n–3 by the 45 elongases At3g06470.

Example 52

Cloning an Elongase from Phaeodactylum tricornutum

Starting from conserved regions in the protein sequences, degenerate primers were constructed with the aid of the elongase genes with $\Delta 6$ -elongase activity detailed in the application, and these primers were Used for searching a Phaeodactylum cDNA library by means of PCR. The following primer sequences were employed:

Name	Sequence	Corresponding
of primer	5'-3'orientation	amino acids
Phaelo forward 1	AA (C/T) CTUCTUTGGCTUTT (C/T) T A (SEQ ID NO: 185)	NLLWLFY (SEQ ID NO: 254)

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-continued

Name	Sequence	Corresponding
of primer	5'-3'orientation	amino acids
Phaelo reverse 1	$ \begin{array}{l} {\rm GA}\left({\rm C}/{\rm T}\right){\rm TGUAC}\left({\rm A}/{\rm G}\right){\rm AA}\left({\rm A}/{\rm G}\right){\rm AA} \\ \left({\rm C}/{\rm T}\right){\rm TGUG}\left({\rm A}/{\rm G}\right){\rm AA} \\ \left({\rm SEQ} \ {\rm ID} \ {\rm NO}: \ 186\right) \end{array} $	FAQFFVQS (SEQ ID NO: 255)

Nucleotide bases in brackets mean that a mixture of oligonucleotides with in each case one or the other nucleotide base are present.

Construction of the Phaeodactylum cDNA Library:

A 21 culture of P. tricornutum UTEX 646 was grown in f/2 medium (Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. In Culture of Marine Invertebrate Animals (Eds. Smith, W. L. and Chanley, M. H.), Plenum Press, New York, pp 29-60) for 14 d (=days) at a light intensity of 35 E/cm². After centrifugation, frozen 20 cells were ground to a fine powder in the presence of liquid nitrogen and resuspended in 2 ml of homogenization buffer (0.33 M sorbitol, 0.3 M NaCl, 10 mM EDTA, 10 mM EGTA, 2% SDS, 2% mercaptoethanol in 0.2 M Tris-Cl pH 8.5). After 4 ml of phenol and 2 ml of chloroform had been added, ²⁵ the mixture was shaken vigorously for 15 minutes at 40-50° C. Thereafter, the mixture was centrifuged (10 min×10 000 g) and the aqueous phase was extracted stepwise with chloroform. Nucleic acids were then precipitated by addition of 1/20 volume 4 M sodium hydrogencarbonate solution and centrifuged. The pellet was taken up in 80 mM Tris-borate pH 7.0 and 1 mM EDTA, and the RNA was precipitated with 8 M lithium chloride. After centrifugation and washing with 70% strength ethanol, the RNA pellet was taken up in RNase-free water. Poly(A)-RNA was isolated using Dyna-³⁵ beads (Dynal, Oslo, Norway) following the manufacturer's instructions, and the first-strand cDNA synthesis was carried out using MLV-Rtase from Roche (Mannheim). Then, the second-strand synthesis was carried out using DNA polymerase I and Klenow fragment, followed by a digestion with RNaseH. The cDNA was then treated with T4 DNA polymerase, and EcoRI/XhoI adaptors (Pharmacia, Freiburg) were subsequently attached by means of T4 ligase. After digestion with XhoI, phosphorylation and gel separation, fragments greater than 300 bp were ligated into the phage lambda ZAP Express following the manufacturer's instructions (Stratagene, Amsterdam, the Netherlands). Following bulk excision of the cDNA library and plasmid recovery, the plasmid library was transformed into E. coli DH10B cells and employed for the PCR screening.

Using the abovementioned degenerate primers, it was possible to generate the PCR fragment with the sequence number SEQ ID NO: 187.

This fragment was labeled with digoxigenin (Roche, Mannheim) and used as probe for screening the phage library.

With the aid of the sequence SEQ ID NO: 187, it was possible to obtain the gene sequence SEQ ID NO: 183, which constitutes the full-RNA molecule of the Phaeodactylum $\Delta 6$ -elongase:

Example 53

Cloning Expression Plasmids for the Heterologous Expression in Yeasts

The relevant primer pairs were selected in such a way that they bore the yeast consensus sequence for highly efficient

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translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the PtELO6 DNAs was carried out in each case using 1 μ l of cDNA, 200 μ M of dNTPs, 2.5 U Advantage polymerase and 100 pmol of each primer in a total volume of 50 μ l. The PCR conditions were as follows: first denaturation for 5 minutes at 95° C., followed by 30 cycles of 30 seconds at 94° C., 1 minute at 55° C. and 2 minutes at 72° C., and a last elongation step of 10 minutes at 72° C.

To clone the sequence for the heterologous expression in yeasts, the following oligonucleotides were used for the PCR reaction:

Name of gene and SEQ ID NO:	
PtELO6 (SEQ ID NO: 183)	F:5'-GCGGCCGCACATAATGATGGTACCTTCAA G (SEQ ID NO: 188)
	R: 3'-GAAGACAGCTTAATAGACTAGT (SEQ ID NO: 189)

*F = foward primer, R = reverse primer

The PCR products-were incubated for 30 minutes at 21° 25 C. with the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. The PCR product (see SEQ ID NO: 192) was ligated into the vector by means of a T overhang and activity of a topoisomerase (Invitrogen). After incubation, E. coli DH5 α cells were 30 transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by means of Qiagen DNAeasy Kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the 35 blank vector pYES2.1 was transformed in parallel. The yeasts were subsequently plated onto complete uracil dropout minimal medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1 and pYES2.1-PtELO6. After the selection, in each case two 40 transformants were selected for further functional expression.

Example 54

Cloning Expression Plasmids for the Seed-Specific Expression in Plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI 50 cleavage sites are introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:

> PSUN-PtELO6 55 Forward: (SEQ ID NO: 190) 5'-GCGGCCGCACCATGATGGTACCTTCAAGTTA Reverse:

(SEQ ID NO: 191) 3'-GAAGACAGCTTAATAGGCGGCCGC

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

- 5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂
- 5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase

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The Advantage polymerase from Clontech was employed. 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 products are incubated with the restriction enzyme NotI for 16 hours at 37° C. The plant expression vector pSUN300-USP is incubated in the same manner. ¹⁰ Thereafter, the PCR products and the 7624 bp vector are separated by agarose gel electrophoresis and the corresponding DNA fragments are excised. The DNA is purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products are ligated. The Rapid Ligation Kit from Roche is used for this purpose. The resulting plasmids pSUN-PtELO is verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, 20 P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Octopine synthase gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene).

(Primer sequence:

(SEQ ID NO: 151) 5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCCGGATC

TGCTGGCTATGAA-3';).

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The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of *Arabidopsis thaliana*, oilseed rape, tobacco and linseed.

Lipids were extracted from yeasts and seeds as described for Example 6.

Example 55

Expression of PtElo in Yeasts

Yeasts which had been transformed with the plasmids pYES2 and pYES2-PtELO6 as in Example 4 were analyzed 60 as follows:

The yeast cells from the main cultures were harvested by centrifugation ($100 \times g$, 5 min, 20° C.) and washed with 100 mM NaHCO₃, pH 8.0 in order to remove residual medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80° C. with 2 ml of 1N methanolic sulfuric acid and 2% (v/v)

dimethoxypropane. The FAMEs were extracted by twice extracting with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM NaHCO₃, pH 8.0, and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4 , evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50° C. to 250° C. with an increment of 5° C./min and finally 10 minutes at 250° C. (holding).

The signals were identified by comparing the retention 15 times with corresponding 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- 20 298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 56

Functional Characterization of PtELO6

FIG. **29** represents the conversion of C18:3^{Δ 6,9,12} and C18.4^{Δ 6,9,12,15}. The substrates are elongated by in each case two carbon atoms; this results in the fatty acids $C20:3^{\Delta 8,11,14}$ and C20:4 $^{\Delta 8,11,14,17}$, respectively. The substrate specificity of PtELO6 can be determined after expression and the feeding of different fatty acids (FIG. 30). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts demonstrated the synthesis of novel fatty acids, the products of the PtElo6 reaction. This means that the gene PtEIO6 has been expressed functionally.

It can be seen from Table 21 that PtElo6 shows a narrow 40 substrate specificity. PtELO6 was only capable of elongating the C18-fatty acids linoleic acid, linolenic acid, y-linolenic acid and stearidonic acid, but preferred the w3-desaturated stearidonic acid (see also FIG. 30).

Feeding experiment: fatty acid's (in bold) were added in ⁴⁵ each case in amounts of 250 µM. The underlined fatty acids were formed de novo.

TABLE 21

			Fatty	acid fed:	
		+18:2	+18:3	+18:3	+18:4
5:0	16.2	18.2	15.2	20	04:48
6:1	50.6	20.5	22.8	33.5	34.2
8:0	5.4	6.3	6.2	5.2	12.4
8:1	27.7	14.6	19.6	19.3	16.7
8:2		40			
8:3			32.9		
8:3				12.3	
8:4					4.5
20:2		0.4			
20:3			<u>3.4</u>		
20:3				9.7	
0:4				_	14.5
% elongation	0.0	0.99	9.37	44.09	76.32

The following fatty acids were fed, but not converted: $\begin{array}{c} & \text{18:1}^{\Delta 6}, 18:1^{\Delta 9}, 18:1^{\Delta 11} \\ 20:2^{\Delta 11,14}, 20:2^{\Delta 11,14} \end{array}$

 $\begin{array}{c} 22^{\Delta 11,14}, & 20:3^{\Delta 11,14,17}, \\ 20:5^{\Delta 5,8,11,14,17} \end{array},$ $20:3^{\Delta 8,11,14}$. $20:4^{\Delta 5,8,11,14}$

 $22:4^{\overline{\Delta7},10,13,16}$

The veasts which had been transformed with the vector pYES2-PtELO6 were cultured in minimal medium in the presence of the fatty acids detailed. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. The results shown in FIGS. 29 and 30 and in Table 19 were thus determined.

Example 57

Cloning Expression Plasmids for the Seed-Specific Expression in Plants

The general conditions described hereinbelow apply to all of the subsequent experiments, unless otherwise specified.

The following are preferably used in accordance with the invention for the examples which follow: Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use is found in Hellens et al., Trends in Plant Science (2000) 5, 446-451. A pGPTV derivative as described in DE10205607 was used. This vector differs from pGPTV by an additionally inserted AscI restriction cleavage site.

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

Cnl1 C

(SEQ ID NO: 203) 35 5':gaattcggcgcgccgagctcctcgagcaacggttccggcggtataga

gttgggtaattcga

```
Cnl1 C
```

(SEO ID NO: 204)

3':cccqqqatcqatqccqqcaqatctccaccattttttqqtqqtqat

Composition of the PCR Mix (50 µl):

- 5.00 µl template cDNA
- 5.00 µl 10× buffer (Advantage polymerase)+25 mM MgCl₂

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl 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 with the restriction enzyme EcoRI for 2 hours at 37° C. and then for 12 hours at 25° C. with the restriction enzyme Smal. The cloning 55 vector pUC19 was incubated in the same manner. Thereafter, the PCR product and the cut, 2668 bp vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1-C was verified by sequencing.

In the next step, the OCS terminator (Genbank Accession V00088; De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti

plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)) from the vector pGPVT-USP/ OCS

(DE 102 05 607) was amplified using the following primers:

(SEQ ID NO: 205) OCS_C 5':aggcctccatggcctgctttaatgagatatgcgagacgcc

> 10(SEQ ID NO: 206)

```
OCS C 3':cccgggccggacaatcagtaaattgaacggag
```

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 μ l 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 15 Composition of the PCR Mix (50 μ l): 5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl 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 with the restriction enzyme StuI for 2 hours at 37° C. and then for 12 hours at 25 25° C. with the restriction enzyme SmaI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25° C. with the restriction enzyme SmaI. Thereafter, the PCR product and the cut vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The 30 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 35 plasmid pUC19-Cnl1-C_OCS was verified by sequencing.

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

Cnl1-B (SEQ ID NO: 207) 5':aggcctcaacggttccggcggtatag

Cnl1-B (SEQ ID NO: 208) 3':cccggggttaacgctagcgggcccgatatcggatcccatttttggt

ggtgattggttct

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 50

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl) 0.50 µl 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 with the restriction enzyme Stul for 2 hours at 37° C. and then for 12 hours at 60 25° C. with the restriction enzyme SmaI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25° C. with the restriction enzyme SmaI. Thereafter, the PCR product and the cut vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The 65 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_OCS was verified by sequencing.

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

(SEQ ID NO: 209) OCS2 5':aggcctcctgctttaatgagatatgcgagac

(SEO ID NO: 210)

(SEQ ID NO: 211)

OCS2 3':cccgggcggacaatcagtaaattgaacggag

5.00 µl template cDNA

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

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

²⁰ 0.50 µl 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 with the restriction enzyme Stul for 2 hours at 37° C. 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 the cut 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_OCS2 was verified by sequencing

In the next step, the Cnl1-A promoter was amplified by ⁴⁰ PCR using the following primers:

Cnl1-B

5':aggcctcaacggttccggcggtatagag Cnl1-B (SEQ ID NO: 212) 3':aggccttctagactgcaggcggccgcccgcattttttggtggtgatt

ggt

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Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

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

5.00 µl 2 mM dNTP

55 1.25 µl of each primer (10 pmol/µl) 0.50 µl Advantage polymerase (Clontech)

PCR Reaction Conditions:

Annealing temperature: 1 min 55° C.

Denaturation temperature: 1 min 94° C. Elongation temperature: 2 min 72° C.

Number of cycles: 35

The PCR product was incubated for 2 hours at 37° C. with the restriction enzyme StuI. The vector pUC19-Cnl1-C was incubated for 12 hours at 25° C. with the restriction enzyme SmaI. Thereafter, the PCR product and the cut vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by

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25

(SEQ ID NO: 213)

means of the Qiagen Gel Purification Kit in accordance with the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1C_Cnl1B_Cnl1A_OCS2 was verified by sequencing.

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

OCS2

5':ggcctcctgctttaatgagatatgcga

OCS2

(SEQ ID NO: 214) 15 3':aagcttggcgcgcgcgagctcgtcgacggacaatcagtaaattgaacg

gaga

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

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

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl 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 with the restriction enzyme StuI for 2 hours at 37° C. 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 the cut 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. ⁴⁰ 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_OCS3 was verified by sequencing.

In the next step, the plasmid pUC19- ⁴⁵ Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the $\Delta 6$ -, $\Delta 5$ -desaturase and $\Delta 6$ -elongase. To this end, the $\Delta 6$ -desaturase from *Phytium irregulare* (WO02/26946) was amplified using the following PCR primers:

(SEQ ID NO: 215) D6Des(Pir) 5':agatctatggtggacctcaagcctggagtg

(SEQ ID NO: 216)

D6Des(Pir) 3':ccatggcccgggttacatcgctgggaactcggtgat

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

- $5.00 \ \mu l \ 10 \times buffer \ (Advantage \ polymerase) + 25 \ mM \ MgCl_2$
- 5.00 µl 2 mM dNTP
- 1.25 µl of each primer (10 pmol/µl)
- 0.50 µl 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

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The PCR product was first incubated with the restriction enzyme BgIII for 2 hours at 37° C. and then for 2 hours at 37° C. with the restriction enzyme A/col. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was incubated for 2 hours at 37° C. with the restriction enzyme BgIII and for 2 hours at 37° C. with the restriction enzyme NcoI. Thereafter, the PCR product and the cut 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 Δ 5-desaturase from *Thraustochytrium* ssp: (WO02/26946): To this end, the Δ 5-desaturase from *Thraustochytrium* ssp. was amplified using the follow-20 ing PCR primers:

(SEQ ID NO: 217) D5Des(Tc) 5':gggatccatgggcaagggcagggggccg

(SEQ ID NO: 218) D5Des(Tc) 3':ggcgccgacaccaagaagcaggactgagatatc

Composition of the PCR Mix (50 µl):

5.00 μl template cDNA

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

5.00 μl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

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

In the next step, the plasmid pUC19-Cnl1_d6Des (Pir)_d5Des(Tc) was used for cloning the Δ 6-elongase from *Physcomitrella patens* (WO01/59128), to which end an amplification with the following PCR primers was carried out:

(SEQ ID NO: 219)

60 D6Elo(Pp) 5':gcggccgcatggaggtcgtggagagattctacggtg

(SEQ ID NO: 220)

D6Elo(Pp) 3':gcaaaagggagctaaaactgagtgatctaga

Composition of the PCR Mix (50 µl):

65 5.00 μl template cDNA

5.00 μ l 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 5.00 μ l 2 mM dNTP

1.25 µl of each primer (10 pmol/µl) 0.50 µl 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 with the restriction enzyme Not for 2 hours at 37° C. and then for 2 hours at 37° 10C. 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 the cut 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 20 resulting plasmid pUC19-Cnl1_d6Des(Pir)_d5Des (Tc)_D6Elo(Pp) was verified by sequencing.

The binary vector for the plant transformation was generated 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 ²⁵ 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 cut pGPTV vector were separated by agarose gel electrophoresis and the relevant DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit in accordance with 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, an amplification was performed starting from pUC19-Cnl1C_OCS, using the following primers:

(SEQ ID NO: 221) Cnl1_OCS 5':gtcgatcaacggttccggcggtatagagttg (SEQ ID NO: 222)

Cnl1 OCS 3':gtcgatcggacaatcagtaaattgaacggaga

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase (Clontech)

PCR Reaction Conditions:

Annealing temperature: 1 min 55° C.

Denaturation temperature: 1 min 94° C.

Elongation temperature: 2 min 72° C.

Number of cycles: 35

The PCR product was incubated for 2 hours at 37° C. with the restriction enzyme Sall. The vector pUC19 was incu- 60 bated for 2 hours at 37° C. with the restriction enzyme SalI. Thereafter, the PCR product and the cut 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 in accordance with 65 the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche

was used for this purpose. The resulting plasmid pUC19-Cnl1_OCS was verified by sequencing.

In a further step, the $\Delta 12$ -desaturase gene from *Calendula* officinalis (WO01/85968) was cloned into pUC19-Cnl1_OCS. To this end, d12Des(Co) was amplified using the following primers:

(SEQ ID NO: 223)

D12Des(Co) 5': agatctatgggtgcaggcggtcgaatgc

(SEQ ID NO: 224) D12Des(Co) 3':ccatqqttaaatcttattacqatacc

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl Advantage polymerase (Clontech)

PCR Reaction Conditions:

Annealing temperature: 1 min 55° C.

Denaturation temperature: 1 min 94° C.

Elongation temperature: 2 min 72° C.

Number of cycles: 35

The PCR product was incubated for 2 hours at 37° C. with the restriction enzyme BgIII and subsequently for 2 hours at the same temperature with A/col. The vector pUC19-Cnl1_OCS was incubated in the same manner. Thereafter, the PCR product and the cut 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 in accordance with the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmid pUC19-Cnl1_D12Des(Co) was verified by sequencing. The plasmid pUC19-Cnl1_D12Des(Co) and the plasmid pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp) were incubated for 2 hours at 37° C. with the restriction enzyme Sall. There-40 after, the vector fragment and the vector were separated by agarose gel electrophoresis and the relevant DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit in accordance with 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 plant transformation was gen-5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 50 erated 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. 55 Thereafter, the fragment from pUC19-Cnl1_d6Des (Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co) and the cut pGPTV vector were separated by agarose gel electrophoresis and the relevant DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit in accordance with 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 vector which is suitable for the transformation of plants is pSUN2. To increase the number of expression cassettes present in the vector to more than four, this vector

was used in combination with the Gateway System (Invitrogen, Karlsruhe). To this end, the Gateway cassette A was inserted into the vector pSUN2 in accordance with the manufacturer's instructions as described hereinbelow:

The pSUN2 vector (1 μ g) was incubated for 1 hour with ⁵ the restriction enzyme EcoRV at 37° C. Thereafter, the Gateway cassette A (Invitrogen, Karlsruhe) was ligated into the cut vector by means of 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 subsequently verified by sequencing.

In the second step, the expression cassette was excised from pUC19-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo (Pp) D12Des(Co) by means of AscI and ligated into the vector pSUN-GW, which had been treated in the same manner. The resulting plasmid pSUN-4G was used for further gene constructs.

To this end, a pENTR clone was first modified in accordance with the manufacturer's instructions (Invitrogen). The 20 plasmid pENTR1A (Invitrogen) was incubated for 1 hour at 37° C. with the restriction enzyme EcorI, subsequently treated for 30 minutes with Klenow enzyme and with one 1 µM dNTP mix, and the AscI adaptor (5'-ggcgcgcc; phosphorylated at the 5' terminus, double-stranded) was then 25 ligated into the vector pENTR1A. Into this modified, genes were stepwise inserted into the Cnl cassette as described above and transferred into the pENTR vector via AscI.

The gene TL16y2 from Thraustochytrium ssp. (SEQ ID NO: 83) was transferred into the pSUN-4G vector in the above described manner:

plasmid pUC19-In the next step, the Cnl1C_Cnl1B_Cnl1A_OCS3 was used for cloning the Δ 5-elongase TL16y2. To this end, the Δ 5-elongase from Thraustochytrium ssp. was amplified using the following 35 PCR primers:

(SEQ ID NO: 225) TL16y2 5':agatet atggaegtegtegageagea

(SEQ ID NO: 226) TL16y2 3':ccatggtccggg agaagcagaagaccatctaa

Composition of the PCR Mix (50 µl):

5.00 µl template cDNA

5.00 µl 10× buffer (Advantage polymerase)+ 25 mM MgCl₂ 45 5.00 µl 2 mM dNTP

1.25 µl of each primer (10 pmol/µl)

0.50 µl 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 BgIII and then for 2 hours at 37° 55 C. with the restriction enzyme NcolI. The vector pUC19-Cnl1C_Cnl1B_Cnl1A_OCS3 was incubated for 2 hours at 37° C. with the restriction enzyme BglII and for 2 hours at 37° C. with the restriction enzyme Ncoll. Thereafter, the PCR product and the cut vector were separated by agarose 60 gel electrophoresis and the relevant DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit in accordance with the manufacturer's instructions. Thereafter, vector and PCR product were ligated. The Rapid Ligation Kit from Roche was used for 65 this purpose. The resulting plasmid pUC19-Cnl1_TL16y2 was verified by sequencing. Thereafter, the cassette was

excised using AscI and ligated into an AscI-pretreated pENTR vector. The resulting plasmid pENTR-Cnl1_TL16y2 was then incubated with the vector pSUN-4G in a recombination reaction in accordance with the manufacturer's instructions (Invitrogen). The product gave the vector pSUN-5G, which was used for the transformation of plants.

In a further step, the construct pSUN-8G was generated using the above-described methodology. To this end, 5' and 3' primers for the genes SEQ ID 41, 53, 87 and 113 with the above-described restriction cleavage sites and the first and in each case last 20 nucleotides of the open reading frame were generated, amplified under the standard conditions (see above) and ligated into the vector pENTR-Cnl.

A recombination reaction with the vector pSUN-4G gave rise to the construct pSUN-8G. This vector too was employed for the transformation of plants.

Example 58

Generation of Transgenic Plants

a) Generation of Transgenic Indian Mustard Plants. The Protocol for the Transformation of Oilseed Rape Plants was Used (Modification of the Method of Moloney et al., 1992, Plant Cell Reports, 8:238-242)

To generate transgenic plants, the binary vectors pGPTV-Cnl1 d6Des(Pir) d5Des(Tc) D6Elo(Pp) D12Des(Co),

pSUN-5G and pSUN-8G which had been generated were transformed into Agrobacterium tumefaciens C58C1: pGV2260 (Deblaere et al., 1984, Nucl. Acids Res. 13, 4777-4788). To transform Indian mustard plants, 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. Petioles or hypocotyls of freshly germinated sterile plants (in each case approx. 1 cm²) were incubated for 5-10 minutes with a 1:50 agrobacterial dilution 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. Cultivation was subsequently continued at 16 hours light/8 hours dark and in a weekly rhythm on MS medium supplemented with 500 mg/l of 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 formed after three weeks, 50 2-indolebutyric acid was added to the medium for rooting, to act as growth hormone.

Regenerated shoots were maintained on 2MS medium supplemented with kanamycin and Claforan, after rooting, transferred into soil and, after cultivation, grown for two weeks in a controlled-environment cabinet or in a greenhouse, allowed to flower, mature seeds were harvested and studied for elongase expression such as $\Delta 6$ -elongase activity or $\Delta 5$ - or $\Delta 6$ -desaturase activity by means of lipid analyses. In this manner, lines with elevated contents of C20- and C22-polyunsaturated fatty acids were identified.

Transgenic oilseed rape plants were also generated successfully using this protocol.

b) Generation of Transgenic Linseed Plants

The transgenic linseed plants can be generated for example by the method of Bell et al., 1999, In Vitro Cell. Dev. Biol.-Plant. 35(6): 456-465 by means of particle bombardment. Agrobacteria-mediated transformations can be

carried out for example by the method of Mlynarova et al. (1994), Plant Cell Report 13:282-285.

Example 59

Lipid Extraction from Seeds

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 15 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 20 example, Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, pp 89-90 and pp 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and 25 Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", pp 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, pp 1-27, VCH: 35 Weinheim; and Dechow, F. J. (1989) Separation and purification techniques in biotechnology, Noves 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 ⁴⁰ 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, 55 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, ana- 60 lyzing 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, 65 Ed., IRL Press, pp 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).

Unambiguous proof of the presence of fatty acid products can be obtained by analyzing recombinant organisms using standard analytical 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 spectrometry 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 1 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 µm, 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 crushing 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 1 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-dimethoxyoxazolin derivatives (Christie, 1998) by 50 means of GC-MS.

Example 60

Analysis of the Seeds from the Transgenic Plants which have been Generated

Analogously to Example 59, the seeds of the plants which had been transformed with the constructs pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co), pSUN-5G and pSUN-8G were analyzed. FIG. **32** shows the fatty acid spectrum of seeds with the construct pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co). In comparison with control plants which were not transformed (wild-type control, WT), a pronounced change in the fatty acid spectrum was observed. It was thus possible to demonstrate that the transformed genes are functional, Table 22 compiles the results of FIG. **32**.

				F	atty acio	is			
Lines	16:0	18:0	18:1	18:2	GLA	18:3	SDA	ARA	EPA
WT	5.6	6.5	31.7	41.7	nd	12.1	nd	nd	nd
control									
1424_Ko82_4	6.6	1.5	8.9	10.5	42.2	3.1	2.8	17.2	0.2
1424_Ko82_5	6.1	1.5	11.0	9.0	40.6	2.9	4.0	15.0	1.5
1424_Ko82_6	5.7	1.6	15.5	10.6	37.1	3.0	3.2	14.6	0.2
1424_Ko82_7	5.4	2.0	20.4	10.7	32.6	3.5	3.2	12.1	1.0
1424_Ko82_8	5.4	1.4	15.1	12.5	39.9	2.6	2.4	12.2	0.7
1424_Ko82_9	6.0	1.8	25.0	9.9	29.7	2.2	2.5	10.2	0.8
1424_Ko82_10	5.7	1.3	10.1	10.3	42.5	2.6	3.5	13.9	1.1
1424_Ko82_11	5.4	1.4	15.7	11.3	38.2	2.6	2.8	14.1	1.0

Here, the analysis of the seeds with the construct pSUN-5G reveals lines with a pronounced increase in the arachidonic acid content in comparison with the construct pGPTV-Cnl1_d6Des(Pir)_d5Des(Tc)_D6Elo(Pp)_D12Des(Co). In ²⁵ this context, lines with up to 25% ARA were obtained. The additional elongase (TL16y2) must be responsible for this effect (FIG. **31**, pSUN-5G). The results from this line are compiled in Table 23.

these seeds. FIG. **32** shows the chromatogram with the modified fatty acid spectrum in comparison with an untransformed control plant. The results of several measurements are compiled in Table 24.

Table 24 shows the fatty acid analysis of transgenic seeds which have been transformed with the construct pSUN-8G.

In this experiment, the synthesis of docosahexaenoic acid in seeds was demonstrated for the first time. While the

TABLE 23

				the co	onstruct	pSUN-	5G.								
		Fatty acids													
Lines	16:0	18:0	18:1	18:2 LA	18:3 GLA	18:3 ALA	18:4 SDA	20:3 HGLA	ARA	EPA					
WT	5.2	2.3	34.2	37.9	0.0	11.6	0.0	0.0	0.0	0.0					
16-1-2	4.2	1.6	20.1	21.5	25.9	4.1	1.8	1.7	8.9	0.8					
16-1-3	5.8	2.3	9.9	14.6	33.6	3.1	2.2	2.2	16.0	1.4					
16-1-8	5.0	2.8	11.1	12.6	34.9	2.2	1.8	2.6	16.3	1.2					
16-2-1	4.9	1.6	14.5	17.4	32.9	3.5	2.0	1.6	12.3	1.0					
16-2-5	5.5	3.3	12.9	13.8	32.9	2.9	2.2	1.4	15.4	1.4					
16-4-2	5.8	2.5	18.8	14.7	32.0	3.5	2.3	1.2	12.0	1.2					
16-4-3	5.9	2.0	19.7	15.0	32.0	3.8	2.4	1.1	11.4	1.2					
16-7-2	6.2	4.4	14.3	10.2	30.7	2.0	2.1	1.7	19.4	1.9					
16-7-3	5.0	2.5	21.6	13.6	30.7	2.1	1.8	1.5	12.6	1.1					
16-7-4	5.3	4.1	18.8	19.5	23.1	4.2	2.2	2.9	11.3	1.4					
16-7-5	7.4	1.8	4.2	6.8	33.7	1.8	2.7	2.6	25.8	2.6					

Example 61

Detection of DHA in Seeds of Transgenic Indian Mustard Plants

Seeds of plants which had been generated with the construct pSUN-8G as described in Example 58 were analyzed as described in Example 59. Besides the LCPUFAs arachidonic acid and eicosapentaenoic acid, docosahexaenoic acid, the product after conversion by the Δ 4-desaturase 65 from *Thraustochytrium* and Δ 5-elongases from *Onchorynchis mykiss* and *Ostreococcus tauri*, was also detected in

synthesis of DHA in higher plants has been described, for example in WO 2004/071467, the synthesis has not been demonstrated for seeds, only for an embryogenic cell culture.

EQUIVALENTS

Many equivalents of the specific embodiments according to the invention described herein can be seen or found by the skilled worker by simple routine experiments. These equivalents are intended to be included in the patent claims.

55

				Т	ABLE 2			
	Fa	atty aci	d distribution	1 in the seeds o	f the three differ	ent transgenic .	B. juncea lines	
<i>B. juncea</i> lines	No.	18:1	18:2 (LA)	γ18:3 (GLA)	α18:3 (ALA)	18:4 (SDA)	20:3 (HGLA)	20:4 (ARA)
WT	1	33.2	38.2	0	12.2	0	0	0
	2	31.3	41.2	0	11.7	0	0	0
8-1424-5	1	25.1	12.8	26.4	3.5	2.4	0.6	8.3
	2	26	12.7	26.3	3.8	2.6	0.6	8.2
	3	25	12.5	25.9	3.4	2.4	0.8	8.5
8-1424-8	1	28.1	13.1	25	5.8	3.7	0.2	6.2
	2	24.7	14.8	26.4	5.2	3	0.3	6.8
8-1424-10	1	25.2	14.2	29.8	5.2	3.4	0.5	5
	2	27.2	12.7	27.9	4.2	2.9	0.3	6.3

The amounts of fatty acids were stated in % by weight.

LA = linoleic acid,

 $GLA = \gamma$ -linolenic acid,

ALA = α -linolenic acid,

SDA = stearidonic acid,

HGLA = dihomo-γ-linolenic acid,

ARA = arachidonic acid,

ETA = eicosatetraenoic acid,EPA = eicosapentaenoic acid

TABLE 3

			Fatty	acid distr	ibution in th	e seeds of th	e three differe	nt transgenic	<i>B. juncea</i> line	5	
Sample	No.	18:1 ∆9	18:2 ∆6, 9	18:2 Δ9, 12 (LA)	18:3 Δ6, 9, 12 (GLA)	18:3 Δ9, 12, 15 (ALA)	18:4 Δ6, 9, 12, 15 (SDA)	20:3 Δ8, 11, 14 (HGLA)	20:4 Δ5, 8, 11, 14 (ARA)	20:4 Δ8, 11, 14, 17 (ETA)	20:5 Δ5, 8, 11, 14, 17 (EPA)
WT	1	35.10	0.00	35.71	0.00	10.80	0.00	0.00	0.00	0.00	0.00
	2	27.79	0.00	32.83	0.00	8.94	0.71	0.00	0.00	0.00	0.00
9-1424-1	1	17.62	1.07	12.32	29.92	2.84	2.17	0.97	13.05	< 0.01	1.21
	2	23.68	2.17	10.57	23.70	2.39	1.80	0.98	11.60	< 0.01	1.16
	3	17.15	0.94	12.86	31.16	3.19	2.40	1.01	12.09	< 0.01	1.16
9-1424-5	1	16.48	1.47	11.09	30.49	3.06	2.56	0.75	11.84	< 0.01	1.24
	2	17.70	1.23	11.42	27.94	2.35	1.88	0.64	12.30	0.03	1.12
	3	19.29	1.05	10.95	26.11	2.85	2.11	1.07	12.09	< 0.01	1.21
9-1424-6	1	24.71	0.00	41.87	0.00	12.32	0.00	0.00	0.00	0.00	0.00
	2	28.84	0.00	40.65	0.00	10.94	0.00	0.00	0.00	0.00	0.00
	3	29.28	0.00	41.34	0.00	10.76	0.00	0.00	0.00	0.00	0.00
9-1424-7	1	32.41	0.00	37.26	0.00	10.05	0.00	0.00	0.00	0.00	0.00
	2	27.76	0.00	36.66	0.00	11.43	0.00	0.00	0.00	0.00	0.00
	3	32.03	0.00	36.27	0.00	9.27	0.00	0.00	0.00	0.00	0.00
9-1424-8	1	19.08	0.61	11.26	23.31	3.73	2.14	1.11	10.93	0.08	1.11
	2	20.34	3.78	10.07	19.59	2.36	1.72	0.68	8.21	< 0.01	1.00
	3	28.27	0.00	37.19	0.00	9.32	0.00	0.00	0.00	0.00	0.00
9-1424-9	1	25.95	0.00	37.87	0.00	9.15	0.00	0.00	0.00	0.00	0.00
	2	22.94	0.00	42.69	0.00	9.14	0.00	0.00	0.00	0.00	0.00
	3	18.96	0.61	14.09	23.76	3.17	1.86	0.97	10.46	< 0.01	0.94

The amounts of fatty acids were stated in % by weight.

LA = linoletic acid, GLA = γ -linoletic acid, ALA = α -linoletic acid,

ALA = α-Informa caid, SDA = stearidonic acid, HGLA = dihomo-y-linolenic acid, ARA = arachidonic acid, ETA = eicosatetraenoic acid, EPA = eicosapentaenoic acid

TABLE	4
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	Fatty acid analysis in seeds of Brassica juncea															
	16:0	18:0	18:1c9	18:1c11	18:2c6, 9	LA 18:2	GLA 18:3	ALA 18:3		20:0	20:1c5	20:2 c8, 11	HGLA 20:3 c8, 11, 14	ARA 20:4		EPA 20:5
WT 16-1-2	5.2 4.2	2.3 1.6	34.2 20.1	3.2 2.3	0.0 0.1	37.9 21.5	0.0 25.9	11.6 4.1	0.0 1.8	0.4 0.4	1.1 1.5	3.7 3.9	0.0 1.7	0.0 8.9	0.0 0.5	0.0 0.8

TABLE 4-continued

					Fa	atty acid an	alysis in	seeds o	f <i>Brass</i>	ica jun	cea					
	16:0	18:0	18:1c9	18:1c11	18:2c6, 9	LA 18:2	GLA 18:3	ALA 18:3	SDA 18:4	20:0	20:1c5	20:2 c8, 11	HGLA 20:3 c8, 11, 14	ARA 20:4	ETA 20:4	EPA 20:5
16-1-3	5.8	2.3	9.9	2.7	0.1	14.6	33.6	3.1	2.2	0.6	1.0	3.2	2.2	16.0	0.4	1.4
16-1-8	5.0	2.8	11.1	2.1	0.3	12.6	34.9	2.2	1.8	0.6	1.3	3.7	2.6	16.3	0.4	1.2
16-2-1	4.9	1.6	14.5	2.9	0.2	17.4	32.9	3.5	2.0	0.4	0.9	1.6	1.6	12.3	1.9	1.0
16-2-5	5.5	3.3	12.9	3.0	0.4	13.8	32.9	2.9	2.2	0.7	1.0	2.2	1.4	15.4	0.3	1.4
16-4-2	5.8	2.5	18.8	2.6	0.9	14.7	32.0	3.5	2.3	0.7	0.8	0.6	1.2	12.0	0.1	1.2
16-4-3	5.9	2.0	19.7	2.5	1.1	15.0	32.0	3.8	2.4	0.5	0.8	0.5	1.1	11.4	0.1	1.2
16-7-2	6.2	4.4	14.3	2.2	0.7	10.2	30.7	2.0	2.1	0.9	0.9	2.1	1.7	19.4	0.3	1.9
16-7-3	5.0	2.5	21.6	1.7	1.5	13.6	30.7	2.1	1.8	0.6	1.1	2.0	1.5	12.6	0.2	1.1
16-7-4	5.3	4.1	18.8	2.2	0.7	19.5	23.1	4.2	2.2	0.7	1.0	1.8	2.9	11.3	0.3	1.4
16-7-5	7.4	1.8	4.2	3.9	0.0	6.8	33.7	1.8	2.7	0.8	0.8	3.2	2.6	25.8	0.6	2.6

The amounts of fatty acids were stated in % by weight.

LA = linoleic acid,

 $GLA = \gamma$ -linolenic acid,

ALA = α -linolenic acid,

SDA = stearidonic acid,

HGLA = dihomo-y-linolenic acid,

ARA = arachidonic acid,

ETA = eicosatetraenoic acid, EPA = eicosapentaenoic acid

TABLE 6	5
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Conversion rates of the fatty acids which have been fed. The conversion rates were calculated using the formula [conversion rate] = [product]/[[substrate] + [product]] * 100 BioTaur clones area in % of the GC analysis														
Clone	fatty acid	C16:0	C16:1 (n-7)	C18:0	C18:1 (n-9)	C18:3 (n-6)	C18:4 (n-3)	C20:3 (n-6)	C20:4 (n-6)	C20:4 (n-3)	C20:5 (n-3)	C22:4 (n-6)	C22:4 (n-3)	C22:5 (n-3)
Vector	none	21.261	41.576	4.670	25.330									
BioTaur	none	20.831	37.374	4.215	26.475									
Vector	GLA + EPA	22.053	23.632	5.487	17.289	11.574					13.792			
BioTaur	GLA + EPA	20.439	25.554	6.129	19.587	3.521		6.620			10.149			1.127
Vector	EPA	20.669	28.985	6.292	21.712						16.225			
BioTaur	EPA	20.472	26.913	6.570	23.131						11.519			3.251
Vector	ARA	23.169	23.332	6.587	12.735				27.069					
BioTaur	ARA	20.969	31.281	5.367	21.351				9.648			1.632		
Vector	SDA	18.519	12.626	6.642	6.344		47.911							
BioTaur	SDA	19.683	15.878	7.246	8.403		13.569			25.946			0.876	

TABLE 24

ſ	16:0	18:0	18:1 Δ9	LA 18:2 Δ9, 12	GLA 18:3 Δ6, 9, 12	ALA 18:3 Δ9, 12, 15	SDA 18:4 ∆6, 9, 12, 1	HGLA 20:3 5 Δ8, 11, 14	ARA 20:4 Δ5, 8, 11, 14	EPA 20:5 Δ5, 8, 11, 14, 17	22:5 ∆7, 10, 13, 16, 19	DHA 22:6 ∆4, 7, 10 13, 16, 19	
WT	5.26	1.80	30.78	43.93	nd	12.47	nd	nd	nd	nd	nd	nd	
Bj-17-1-3	4.73	2.28	19.30	14.04	31.48	3.09	2.40	1.70	3.37	8.65	0.19	0.25	
Bj-17-2-1	4.34	2.17	17.60	15.56	29.97	3.37	2.44	2.14	4.05	9.14	0.23	0.40	
Bj-17-4-3	4.31	1.70	14.45	16.94	35.54	3.43	2.39	0.10	5.09	9.43	0.24	0.23	
Π	I			% aturated tty acids		% mono- unsaturated fatty acids	u	% poly- nsaturated atty acids	% LC	FAs	% VLCFA	s	
W	Τ		7.96 9.18 9.83			35.43		56.62	97.7	1	2.29		
В	j-17-1-3					24.95		65.87	79.6	4	20.36		
В	j-17-2-1					25.44		64.73	80.4	4	19.56		
Bj-17-4-3				14.05		20.36		65.60	75.2	7	24.73		

LCFAs = all fatty acids up to a length of 18 carbon atoms in the fatty acid chain

VLCFAs = all fatty acids with a length of 20 or more carbon atoms in the fatty acid chain

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105 of 290

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Ile	Glu	Asn 35	Tyr	Gln	Gly	Arg	Asp 40	Ala	Thr	Asp	Ala	Phe 45	Met	Val	Met		
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													τın							
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Gly	His	Asp 195	Pro	Asp	Ile	Asp	Asn 200	Leu	Pro	Leu	Leu	Ala 205	Trp	Ser	Glu					
Asp	Asp 210	Val	Thr	Arg	Ala	Ser 215	Pro	Ile	Ser	Arg	Lys 220	Leu	Ile	Gln	Phe					
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US 9,458,436 B2

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165170175Thr Ala Ala Cly Tyr Lys Phe Lys Ala Lys Pro Leu IIe 180Thr Ala Mat 190Thr Ala Mat 190Gln IIe Cys Gln Phe Val Gly Gly Phe Leu Leu Val Trp Asp Tyr IIe 210Phe Asn Ser Asp Lys Gly Lys Leu Phe Ser Trp Ala 220Ann Val Pro Cys Phe Asn Ser Asp Lys Gly Lys Leu Phe Ser Trp Ala 215Phe Asn Tyr Ala Tyr Val Gly Ser Val Phe Leu Leu Phe Cys His Phe 225Phe Asn Tyr Ala Tyr Val Gly Ser Val Phe Leu Leu Phe Cys His Phe 245Phe Cys Phe Asn Ser Asp Lys Ser Ala Lys Ala Gly Lys 250Color Low SEO ID NO 5 (212) TPE DNA (213) ORGANISM: Phaeodactylum tricornutum (220) FEATURE: (222) LOCATION: (1)(1410) (221) NMAK/KSY: CDS (222) LOCATION: (1)(1410) (222) TPE INFORMATION: Delta-5 desaturase<400> SEQUENCE: 5at gg ct ccg gat gcg gat asg ctt cga caa cgc cag acg act gcg gta (20) SEQUENCE: 5at gg ct ccg gat gcg cg as asg ctt cga cac cg cag acc act tyc agt (20) Ser 20)ct gc at act gct gct acc att tog acg cag gaa cgc ctt tyc agt (20) 25ct gc at act gct gct acc att cg acg cag gaa cdc act ct tt (20) 400ct gc ct caa tc dt cg at cac ccc ggg gg gaa acg atc aca atg ttt (20) 400gac ct ca ca tact tc gat cat ccc ggg gg gaa acg atc aaa atg ttt (20) 400gg cg cac gat gct cd cg ta cd at cac gcg gcg ad cac act tfor (20) 400gg cg cac gat gct cd gt cac tyca cag tac aag atg atc acc ccc tac (20) 400gg cg cac ag tg tc act gta ca ccc gad gt gcg gaa dcg acg gaa cac at (20) 400gg cg cac gat gtc act gta cac ccc gad gt gcg gaa act aca (20) 400gg cg cac gat gt cac gt cac gt cac ag tac aag atg atc acc ccc act (20) 400gg tu ct cc aa tact tt gaa aag tg acg gd gtt	Val 145	Tyr	Leu	Gly	Ile	-	Leu	His	Asn	Glu		Val	Trp	Ile	Phe		
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195200205Asn Val Pro Cys Phe Asn Ser Asp Lys Gly Lys Leu Phe Ser Trp Ala 215215Phe Asn Tyr Ala Tyr Val Gly Ser Val Phe Leu Phe Cys His Phe 225225Phe Tyr Gln Asp Asn Leu Ala Thr Lys Lys Ser Ala Lys Ala Gly Lys 245210SEQ ID NO 5 (211> LENGTH: 1410 (212> TYPE: DNA<210> SEQ ID NO 5 (211> LENGTH: 1410 (212> TYPE: DNA<210> SEQ ID NO 5 (211> LENGTH: 1410 (212> TYPE: DNA<220> NAME/KEY: COS (222> LOCATION: (1)(1410) (223> COTHER INFORMATION: Delta-5 desaturase<400> SEQUENCE: 5atg gct ccg gat gcg gat asg ctt cga caa cgc cag acg act gcg gta Met Ala Pro Asp Ala Asp Lys Leu Arg Gln Arg Gln Thr Thr Ala Val 115gcg aag cac aat gct gct acc at tcg acg cag cag cgc ctt tgc agt 20Ala Lys His Asn Ala Ala Thr ILe Ser Thr Gln Glu Arg Leu Cys Ser 20ctg ct ccg dat gct gct acc at tcg agt cag dat cat c tat 20144 Leu Ser Ser Leu Lys Gly Gly Glu Glu Val Cys ILe App Gly ILe II e Tyr 3540041042142142142142242342442442542442642742842942942942042042142142142442042142142142142442542042142142142	Thr	Ala	Ala	-	Tyr	Lys	Phe	Lys		Lys	Pro	Leu	Ile		Ala	Met	
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225 230 235 240 Phe Tyr Gln Asp Asn Leu Ala Thr Lys Lys Ser Ala Lys Ala Gly Lys 245 250 255 Gln Leu 210 5250 215 c210> SEQ ID NO 5 2215 255 c211> LENGTH: 1410 2215 255 c212> ANAK/KEY: CDS 2215 2222 c221> NAK/KEY: CDS 2223 c221> NAK/KEY: CDS 2223 c222> LOCATION: (1)(1410) 2223 c222> LOCATION: (1)(1410) 2223 c223> OTHER INFORMATION: Delta-5 desaturase 40 c400> SEQUENCE: 5 310 atd gct ccg gat gcg gat aag ctt ccg aca cac cc cat gcg gat ccg ctt tgc agt 20 hat Lys His Asn Ala Asp Lys Leu Arg Gln Arg Gln Thr Thr Ala Val 15 gcg aag cac aat gct gct acc ata tcg acg cag gaa cgc ctt tgc agt 30 ctg tct tcg ctc aaa gcg gaa gaa gtc tgc atc gac gga atc atc tat 144 Leu Ser Ser Leu Lys Gly Glu Glu Val Cys Ile Asp Gly Ile Ile Tyr 45 gac ctc caa tca ttc gat cat cac cgg ggt gaa acg atc aca atg ttt 245 Asp Leu Gln Ser Phe Ap His Pro Gly Gly Glu Thr Ile Lys Met Phe 55 66 70 70 75 acc gag aag cat ttg gaa aag atg ag cgt gcg gca ag gta cg gat 240	Asn		Pro	Суз	Phe	Asn		Asp	Lys	Gly	Lys		Phe	Ser	Trp	Ala	
Callo SEQ ID NO 5 Callo Leu Callo SEQ ID NO 5 Callo LENGTH: 1410 Callo SEQUENTRE: Callo NAME/KEY: CDS Callo NAME/KEY: CDS Callo CATION: (1)(1410) Callo SEQUENCE: 5 Callo SE	Phe 225	Asn	Tyr	Ala	Tyr		Gly	Ser	Val	Phe		Leu	Phe	Суа	His		
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Thr Glu Lys His Leu Glu Lys Met Lys Arg Val Gly Lys Val Thr Asp 85 90 90 95 90 105 100 105 110 110 110 120 120 125						Thr					Met					His	240
Phe Val Cys Glu Tyr Lys Phe Asp Thr Glu Phe Glu Arg Glu Ile Lys 100 105 110 cga gaa gtc ttc aag att gtg cga cga ggc aag gat ttc ggt act ttg 384 Arg Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu 115 120 gga tgg ttc ttc cgt gcg ttt tgc tac att gcc att ttc ttc tac ctg 432 Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu 432			-		Leu	-	-	-	-	Arg	-		-		Thr	-	288
Arg Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu 115 120 125 125 gga tgg ttc ttc cgt gcg ttt tgc tac att gcc att ttc ttc tac ctg 432 Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu 432		-	-	Glu		-		-	Thr	-		-	-	Glu			336
Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu	-	-	Val		-			Arg	-		-	-	Phe			-	384
		Trp			-		Phe	-			-	Ile				-	432

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-			-	-		-					gac Asp			-	-	720
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											ggt Gly					864
	-				-	-	-	-			gtt Val 300				-	912
											tac Tyr					960
											atg Met					1008
	-	-			-		-	-		-	ttg Leu	-				1056
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											tcc Ser 380					1152
											aac Asn					1200
		-			-	-	~	-	-		tat Tyr				~	1248
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	-										gtc Val	-		-		1344
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Thr	Glu	Lys	His	Leu 85	Glu	Lys	Met	Lys	Arg 90	Val	Gly	Lys	Val	Thr 95	Asp		
Phe	Val	Сув	Glu 100	Tyr	Lys	Phe	Asp	Thr 105	Glu	Phe	Glu	Arg	Glu 110	Ile	Lys		
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Tyr	Gly	Ile	Ser	Gln 165	Ala	Met	Ile	Gly	Met 170	Asn	Val	Gln	His	Asp 175	Ala		
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His	Trp 210	Thr	His	His	Ala	Tyr 215	Thr	Asn	His	Ala	Glu 220	Met	Asp	Pro	Asp		
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					Gln												
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					acc Thr											144	
					ggt Gly											192	
				Cys	cct Pro 70		Gln	Glu		Glu						240	
					gga Gly											288	
				-	tct Ser	-					-			-		336	
-	-	-	-	-	gca Ala	-			-	-				-		384	
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153

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cag ttg														
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atg gtc Met Val														1296
cga aat							ttg Leu						tag	1344

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155

156

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Gly	Ser	Ala 35	Ile	Thr	Thr	Tyr	Lys 40	Asn	Met	Asp	Ala	Thr 45	Thr	Val	Phe
His	Thr 50	Phe	His	Thr	Gly	Ser 55	Lys	Glu	Ala	Tyr	Gln 60	Trp	Leu	Thr	Glu
Leu 65	Lys	Lys	Glu	Суз	Pro 70	Thr	Gln	Glu	Pro	Glu 75	Ile	Pro	Asp	Ile	Lys 80
Asp	Asp	Pro	Ile	Lys 85	Gly	Ile	Asp	Asp	Val 90	Asn	Met	Gly	Thr	Phe 95	Asn
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Arg	Met	Arg 115	Val	Arg	Ala	Glu	Gly 120	Leu	Met	Asp	Gly	Ser 125	Pro	Leu	Phe
Tyr	Ile 130	Arg	Lys	Ile	Leu	Glu 135	Thr	Ile	Phe	Thr	Ile 140	Leu	Phe	Ala	Phe
Tyr 145	Leu	Gln	Tyr	His	Thr 150	Tyr	Tyr	Leu	Pro	Ser 155	Ala	Ile	Leu	Met	Gly 160
Val	Ala	Trp	Gln	Gln 165	Leu	Gly	Trp	Leu	Ile 170	His	Glu	Phe	Ala	His 175	His
Gln	Leu	Phe	Lys 180	Asn	Arg	Tyr	Tyr	Asn 185	Asp	Leu	Ala	Ser	Tyr 190	Phe	Val
Gly	Asn	Phe 195	Leu	Gln	Gly	Phe	Ser 200	Ser	Gly	Gly	Trp	Lys 205	Glu	Gln	His
Asn	Val 210	His	His	Ala	Ala	Thr 215	Asn	Val	Val	Gly	Arg 220	Asp	Gly	Asp	Leu
Asp 225	Leu	Val	Pro	Phe	Tyr 230	Ala	Thr	Val	Ala	Glu 235	His	Leu	Asn	Asn	Tyr 240
Ser	Gln	Asp	Ser	Trp 245	Val	Met	Thr	Leu	Phe 250	Arg	Trp	Gln	His	Val 255	His
Trp	Thr	Phe	Met 260	Leu	Pro	Phe	Leu	Arg 265	Leu	Ser	Trp	Leu	Leu 270	Gln	Ser
Ile	Ile	Phe 275	Val	Ser	Gln	Met	Pro 280	Thr	His	Tyr	Tyr	Asp 285	Tyr	Tyr	Arg
Asn	Thr 290	Ala	Ile	Tyr	Glu	Gln 295	Val	Gly	Leu	Ser	Leu 300	His	Trp	Ala	Trp
Ser 305	Leu	Gly	Gln	Leu	Tyr 310	Phe	Leu	Pro	Asp	Trp 315	Ser	Thr	Arg	Ile	Met 320
Phe	Phe	Leu	Val	Ser 325	His	Leu	Val	Gly	Gly 330	Phe	Leu	Leu	Ser	His 335	Val
Val	Thr	Phe	Asn 340	His	Tyr	Ser	Val	Glu 345	Lys	Phe	Ala	Leu	Ser 350	Ser	Asn
Ile	Met	Ser 355	Asn	Tyr	Ala	Суз	Leu 360	Gln	Ile	Met	Thr	Thr 365	Arg	Asn	Met
Arg	Pro 370	Gly	Arg	Phe	Ile	Asp 375	Trp	Leu	Trp	Gly	Gly 380	Leu	Asn	Tyr	Gln
Ile 385	Glu	His	His	Leu	Phe 390		Thr	Met	Pro	Arg 395		Asn	Leu	Asn	Thr 400
	Met	Pro	Leu			Glu	Phe	Ala			Asn	Gly	Leu		
Met	Val	Asp		405 Tyr	Phe	Thr	Gly		410 Trp	Leu	Glu	Ile		415 Gln	Phe
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CSIRO Exhibit 1013

Arg Asn Ile Ala Asn Val Ala Ala Lys Leu Thr Lys Lys Ile Ala

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gt tgg ttg gtg ata tgg ggc aaa gtc tac gat gtc aca agc tgg As
p Cys Trp Leu Val Ile Trp Gly Lys Val Tyr Asp Val Thr Ser Trp-att ccc aat cat ccg ggg ggc agt ctc atc cac gta aaa gca ggg cag Ile Pro Asn His Pro Gly Gly Ser Leu Ile His Val Lys Ala Gly Gln gat too act cag ott tto gat too tat cao ooc ott tat gto agg aaa Asp Ser Thr Gln Leu Phe Asp Ser Tyr His Pro Leu Tyr Val Arg Lys atg ctc gcg aag tac tgt att ggg gaa tta gta ccg tct gct ggt gat Met Leu Ala Lys Tyr Cys Ile Gly Glu Leu Val Pro Ser Ala Gly Asp gac aag ttt aag aaa gca act ctg gag tat gca gat gcc gaa aat gaa Asp Lys Phe Lys Lys Ala Thr Leu Glu Tyr Ala Asp Ala Glu Asn Glu gat ttc tat ttg gtt gtg aag caa cga gtt gaa tct tat ttc aag agt Asp Phe Tyr Leu Val Val Lys Gln Arg Val Glu Ser Tyr Phe Lys Ser aac aag ata aac ccc caa att cat cca cat atg atc ctg aag tca ttg Asn Lys Ile Asn Pro Gln Ile His Pro His Met Ile Leu Lys Ser Leu ttc att ctt ggg gga tat ttc gcc agt tac tat tta gcg ttc ttc tgg Phe Ile Leu Gly Gly Tyr Phe Ala Ser Tyr Tyr Leu Ala Phe Phe Trp 165 170 175 tet tea agt gte ett gtt tet ttg ttt tte gea ttg tgg atg ggg tte Ser Ser Ser Val Leu Val Ser Leu Phe Phe Ala Leu Trp Met Gly Phe ttc gca gcg gaa gtc ggc gtg tcg att caa cat gat gga aat cat ggt Phe Ala Ala Glu Val Gly Val Ser Ile Gln His Asp Gly Asn His Gly tca tac act aaa tgg cgt ggc ttt gga tat atc atg gga gcc tcc cta Ser Tyr Thr Lys Trp Arg Gly Phe Gly Tyr Ile Met Gly Ala Ser Leu gat cta gtc gga gcc agt agc ttc atg tgg aga cag caa cac gtt gtg Asp Leu Val Gly Ala Ser Ser Phe Met Trp Arg Gln Gln His Val Val gga cat cac tcg ttt aca aat gtg gac aac tac gat cct gat att cgt Gly His His Ser Phe Thr Asn Val Asp Asn Tyr Asp Pro Asp Ile Arg

159

gtg Val																816
tgg Trp																864
cta Leu																912
gga Gly 305					-	-				-			-			960
aac Asn																1008
ttg Leu																1056
tat Tyr		-		-						_		-				1104
caa Gln																1152
999 Gly 385																1200
gat Asp		-		-							-					1248
aac Asn							-					-		-		1296
tat Tyr																1344
gtg Val																1392
ttt Phe 465				Lys		Val		Leu		Glu	Phe			Āsp		1440
tga																1443
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Leu	Arg	Leu	Arg 20	Thr	Ser	Asn	Ser	Lys 25	Gly	Pro	Glu	Gln	Glu 30	Gln	Thr	
Leu	Lys	Lуз 35	Tyr	Thr	Leu	Glu	Asp 40	Val	Ser	Arg	His	Asn 45	Thr	Pro	Ala	
Asp	Cys 50	Trp	Leu	Val	Ile	Trp 55	Gly	Гла	Val	Tyr	Asp 60	Val	Thr	Ser	Trp	

Ile 65	Pro	Asn	His	Pro	Gly 70	Gly	Ser	Leu	Ile	His 75	Val	LÀa	Ala	Gly	Gln 80
Asp	Ser	Thr	Gln	Leu 85	Phe	Asp	Ser	Tyr	His 90	Pro	Leu	Tyr	Val	Arg 95	Lys
Met	Leu	Ala	Lys 100	Tyr	Cys	Ile	Gly	Glu 105	Leu	Val	Pro	Ser	Ala 110	Gly	Asp
Asp	Lys	Phe 115	Lys	Lys	Ala	Thr	Leu 120	Glu	Tyr	Ala	Asp	Ala 125	Glu	Asn	Glu
Asp	Phe 130	Tyr	Leu	Val	Val	Lys 135	Gln	Arg	Val	Glu	Ser 140	Tyr	Phe	Lys	Ser
Asn 145	Lys	Ile	Asn	Pro	Gln 150	Ile	His	Pro	His	Met 155	Ile	Leu	Lys	Ser	Leu 160
Phe	Ile	Leu	Gly	Gly 165	Tyr	Phe	Ala	Ser	Tyr 170	Tyr	Leu	Ala	Phe	Phe 175	Trp
Ser	Ser	Ser	Val 180	Leu	Val	Ser	Leu	Phe 185	Phe	Ala	Leu	Trp	Met 190	Gly	Phe
Phe	Ala	Ala 195	Glu	Val	Gly	Val	Ser 200	Ile	Gln	His	Asp	Gly 205	Asn	His	Gly
Ser	Tyr 210	Thr	Гла	Trp	Arg	Gly 215	Phe	Gly	Tyr	Ile	Met 220	Gly	Ala	Ser	Leu
Asp 225	Leu	Val	Gly	Ala	Ser 230	Ser	Phe	Met	Trp	Arg 235	Gln	Gln	His	Val	Val 240
Gly	His	His	Ser	Phe 245	Thr	Asn	Val	Asp	Asn 250	Tyr	Asp	Pro	Asp	Ile 255	Arg
Val	Lys	Aab	Pro 260	Asp	Val	Arg	Arg	Val 265	Ala	Thr	Thr	Gln	Pro 270	Arg	Gln
Trp	Tyr	His 275	Ala	Tyr	Gln	His	Ile 280	Tyr	Leu	Ala	Val	Leu 285	Tyr	Gly	Thr
Leu	Ala 290	Leu	Lys	Ser	Ile	Phe 295	Leu	Asp	Asp	Phe	Leu 300	Ala	Tyr	Phe	Thr
Gly 305	Ser	Ile	Gly	Pro	Val 310	Lys	Val	Ala	Гуз	Met 315	Thr	Pro	Leu	Glu	Phe 320
Asn	Ile	Phe	Phe	Gln 325	Gly	Lys	Leu	Leu	Tyr 330	Ala	Phe	Tyr	Met	Phe 335	Val
Leu	Pro	Ser	Val 340	Tyr	Gly	Val	His	Ser 345	Gly	Gly	Thr	Phe	Leu 350	Ala	Leu
		355					360					365		Leu	
	370					375	-				380			Glu	-
385	-		-	-	390	_				395				Thr	400
Asp	Phe	Ser	Pro	Arg 405	Ser	Trp	Phe	Trp	Gly 410	His	Val	Ser	Gly	Gly 415	Leu
Asn	Asn	Gln	Ile 420	Glu	His	His	Leu	Phe 425	Pro	Gly	Val	Сүз	His 430	Val	His
Tyr	Pro	Ala 435	Ile	Gln	Pro	Ile	Val 440	Glu	Lys	Thr	Сув	Lys 445	Glu	Phe	Asp
Val	Pro 450	Tyr	Val	Ala	Tyr	Pro 455	Thr	Phe	Trp	Thr	Ala 460	Leu	Arg	Ala	His
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	gcg acg Ala Thr 35												144
	gag ggc Glu Gly												192
	cag cgg Gln Arg		ly Lys										240
	gat gcg Asp Ala				-			-	-			-	288
gcg cgg Ala Arg	cgc gac Arg Asp 100												336
	gcc gag Ala Glu 115												384
	gtg gag Val Glu												432
	gcc tcg Ala Ser		nr Ser										480
	cag ggc Gln Gly												528
	acg ggc Thr Gly 180												576
	gtc ggc Val Gly 195												624
	cac cac His His		-		-				-	-	-		672
-	ctg ccc Leu Pro	Leu Va	-				-	-		-	-	-	720
aag ccg Lys Pro	gga tcg Gly Ser												768
	ccc gtc Pro Val 260												816

165

												COII	CIII	ucu			
	cac His															864	
	atc Ile 290															912	
	tac Tyr	-	-			-	-		-		-	-	-			960	
	ggc Gly															1008	
	ccg Pro															1056	
	gac Asp															1104	
	atg Met 370	-		-			-								-	1152	
	ccg Pro															1200	
	aag Lys	-				-			-	-			~	~	~ ~	1248	
-	tcg Ser				-					-			-	-		1296	
-	gac Asp		-	-	-	-	tga									1320	
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Tyr	Asp	Ala 35	Thr	Asn	Phe	ГЛа	His 40	Pro	Gly	Gly	Ser	Ile 45	Ile	Asn	Phe		
Leu	Thr 50	Glu	Gly	Glu	Ala	Gly 55	Val	Asp	Ala	Thr	Gln 60	Ala	Tyr	Arg	Glu		
Phe 65	His	Gln	Arg	Ser	Gly 70	ГЛа	Ala	Asp	Lys	Tyr 75	Leu	ГЛа	Ser	Leu	Pro 80		
Lys	Leu	Asp	Ala	Ser 85	Lys	Val	Glu	Ser	Arg 90	Phe	Ser	Ala	Lys	Glu 95	Gln		
Ala	Arg	Arg	Asp 100	Ala	Met	Thr	Arg	Asp 105	Tyr	Ala	Ala	Phe	Arg 110	Glu	Glu		
Leu	Val	Ala 115	Glu	Gly	Tyr	Phe	Asp 120	Pro	Ser	Ile	Pro	His 125	Met	Ile	Tyr		
Arg	Val 130	Val	Glu	Ile	Val	Ala 135	Leu	Phe	Ala	Leu	Ser 140	Phe	Trp	Leu	Met		

168

Ser 145	Гла	Ala	Ser	Pro	Thr 150	Ser	Leu	Val	Leu	Gly 155	Val	Val	Met	Asn	Gly 160	
Ile	Ala	Gln	Gly	Arg 165	Cys	Gly	Trp	Val	Met 170	His	Glu	Met	Gly	His 175	Gly	
Ser	Phe	Thr	Gly 180	Val	Ile	Trp	Leu	Asp 185	Asp	Arg	Met	Суз	Glu 190	Phe	Phe	
Tyr	Gly	Val 195	Gly	Суз	Gly	Met	Ser 200	Gly	His	Tyr	Trp	Lys 205	Asn	Gln	His	
Ser	Lys 210	His	His	Ala	Ala	Pro 215	Asn	Arg	Leu	Glu	His 220	Asp	Val	Asp	Leu	
Asn 225	Thr	Leu	Pro	Leu	Val 230	Ala	Phe	Asn	Glu	Arg 235	Val	Val	Arg	Lys	Val 240	
Lys	Pro	Gly	Ser	Leu 245	Leu	Ala	Leu	Trp	Leu 250	Arg	Val	Gln	Ala	Tyr 255	Leu	
Phe	Ala	Pro	Val 260	Ser	CÀa	Leu	Leu	Ile 265	Gly	Leu	Gly	Trp	Thr 270	Leu	Tyr	
Leu	His	Pro 275	Arg	Tyr	Met	Leu	Arg 280	Thr	Lys	Arg	His	Met 285	Glu	Phe	Val	
Trp	Ile 290	Phe	Ala	Arg	Tyr	Ile 295	Gly	Trp	Phe	Ser	Leu 300	Met	Gly	Ala	Leu	
Gly 305	Tyr	Ser	Pro	Gly	Thr 310	Ser	Val	Gly	Met	Tyr 315	Leu	Сув	Ser	Phe	Gly 320	
Leu	Gly	Cys	Ile	Tyr 325	Ile	Phe	Leu	Gln	Phe 330	Ala	Val	Ser	His	Thr 335	His	
Leu	Pro	Val	Thr 340	Asn	Pro	Glu	Asp	Gln 345	Leu	His	Trp	Leu	Glu 350	Tyr	Ala	
Ala	Asp	His 355	Thr	Val	Asn	Ile	Ser 360	Thr	Lys	Ser	Trp	Leu 365	Val	Thr	Trp	
Trp	Met 370	Ser	Asn	Leu	Asn	Phe 375	Gln	Ile	Glu	His	His 380	Leu	Phe	Pro	Thr	
Ala 385	Pro	Gln	Phe	Arg	Phe 390	Lys	Glu	Ile	Ser	Pro 395	Arg	Val	Glu	Ala	Leu 400	
Phe	Lys	Arg	His	Asn 405	Leu	Pro	Tyr	Tyr	Asp 410	Leu	Pro	Tyr	Thr	Ser 415	Ala	
Val	Ser	Thr	Thr 420	Phe	Ala	Asn	Leu	Tyr 425	Ser	Val	Gly	His	Ser 430	Val	Gly	
Ala	Asp	Thr 435	Lys	Lys	Gln	Asp										
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					gga Gly											48
cat				gac	gac Asp				gcc					gtg		96
			20	-	ttg			25			-	-	30		-	144
					Leu											

- 1	<u> </u>
	hU.
	\mathbf{v}

170

											-	con	tin	led			
		35					40					45					
-			-		-	-	-		-	-	ttt Phe 60		-			192	
											tac Tyr					240	
											cca Pro					288	
			-	-	-	-					acg Thr	-				336	
-		-		-						-	tac Tyr	-				384	
											ttt Phe 140					432	
											atc Ile					480	
	<u> </u>	<u> </u>		<u> </u>	00						gat Asp					528	
											ctg Leu					576	
											atg Met					624	
											gca Ala 220					672	
-	-				-	-	-	-		_	ccc Pro			-		720	
											cct Pro					768	
											aac Asn					816	
-	-			-	-		-	-			atc Ile	-				864	
											gtc Val 300					912	
	-		-	-		-		-		-	gtg Val	-		-		960	
											gcg Ala					1008	
											ttg Leu					1056	
<u>aaa</u>	atc	atc	caa	aag	gac	tgg	gca	gct	atg	cag	gtc	gag	act	acg	cag	1104	

Gly	Ile	Ile 355	Gln	Lys	Asp	Trp	Ala 360	Ala	Met	Gln	Val	Glu 365	Thr	Thr	Gln	
~		-		-	tcg Ser					-			~~	~	-	1152
					cac His 390											1200
					gcc Ala											1248
•				•	aag Lys						•		•			1296
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Phe Val Asn His Ile Asn Gln His Met Phe Val Pro Phe Leu Tyr Gly

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Thr Val Ala Asp Met Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln 325 Ala Asn His Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn 340 Gly Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln 355 Ala Asn His Val Val Bu Glu Val Gln Trp Thr Ser Ile Thr Gly Ser Leu 370 Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu 370 Ann Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His 390 Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys 400 Tyr Pro Asp Ile Leu Ala Ile Gly Leu Arg Pro Lys Glu Glu 435 400 Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His 420 Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu 435 440 445 445 445 445 445 445 44												-	COIL		uea		
275 280 285 Phr Val Met Phe Trp Gly Gly Lyz Ala Phe Phe Val Trp Tyr Arg Leu 295 295 (200) 11e Val Pro Leu Gln Tyr Leu Pro Leu Gly Lyg Val Leu Leu Leu Phe 310 330 335 336 1eu Leu Leu Phe 320 336 337 336 An An His Val Val Glu Glu Val Gln Trp Pro Leu Pro Ang Glu Aen 345 336 331 336 365 Sha Aan His Val Val Glu Glu Val Gln Trp Pro Leu Pro Ang Glu Aen 345 365 Shy Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu 370 365 Ang Tyr Gln Ala Val His His Leu Phe Pro Ang Val Ser Glu His His 400 395 Var Pro Aap Ile Leu Ala Ile Ile Lyg Asn Thr Cys Ser Glu Tyr Lys 410 410 420 420 410 410 420 410 440 420 410 440 421 420 440 421 440 445 421 440 445 421 440 445 421 440 445 421 440 445 421 440 445 421 440 445	Leu	Leu	Ala		Lys	Val	Arg	Ile		Asp	Ile	Asn	Ile		Tyr	Phe	
290 295 300 le Val Pro Leu Gu Tyr Leu Gu Su	al	Lys		Asn	Asp	Ala	Ile		Val	Asn	Pro	Ile		Thr	Trp	His	
305 310 315 320 Thr Val Ala App Net Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln 335 Ala Aen His Val Val Glu Glu Val Gln Trp Pro Leu Pro App Glu Aen 346 340 340 Glu Glu Val Gln Trp Pro Leu Pro App Glu Aen 350 375 360 Gly He He Gln Lyg App Trp Ala Ala Met Gln Val Glu Thr Thr Gln 365 Aan Tyr Gln Ala Val His His Leu Phe Pro Aen Val Ser Gln His His 400 770 Yr Leu Ala IIe IIe Lys App Thr Phe Trp Gln Ala Phe Ala Ser His 400 400 797 Pro App IIe Leu Ala IIe IIe Lys App Thr Phe Trp Gln Ala Phe Ala Ser His 410 415 Val Pro Tyr Leu Val Lys App Thr Phe Trp Gln Ala Phe Ala Ser His 420 440 421 440 421 Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu 421 212 NMAR/KST CDS 2213 MARTING Phe Phe Phe Fle ILe Lys App IIe Lys App Ala Val Leu Arg Ser Jin Phe Phe Fle ILe Lys IIe App App Ala Val Leu Arg Ser His Pro Gly 48 4210 10 145 445 445 2210 NARTHET 10 15 10 15 2213 MARTHET <td< td=""><td>Thr</td><td></td><td>Met</td><td>Phe</td><td>Trp</td><td>Gly</td><td></td><td>Lys</td><td>Ala</td><td>Phe</td><td>Phe</td><td></td><td>Trp</td><td>Tyr</td><td>Arg</td><td>Leu</td><td></td></td<>	Thr		Met	Phe	Trp	Gly		Lys	Ala	Phe	Phe		Trp	Tyr	Arg	Leu	
325330335Ala Aan His Val Val Glu Glu Ual Gln Trp Pro Leu Pro App Glu Aan 340345Gly He Hie Gln Lys App Trp Ala Ala Met Gln Val Glu Thr Thr Gln 360366Ala Man Tyr Ala His Asp Ser His Leu Trp Thr Ser He Tr Gly Ser Leu 370370Am Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His 400395Tyr Pro Asp He Leu Ala He He Lus Asn Thr Cys Ser Glu Tyr Lys 405400Yal Pro Tyr Leu Val Lys Asn Thr Phe Trp Gln Ala Phe Ala Ser His 425420Val Pro Tyr Leu Val Lys Asn Thr Pho Lys Glu Glu 435440435440445440445435440 <t< td=""><td>Ile 305</td><td>Val</td><td>Pro</td><td>Leu</td><td>Gln</td><td>-</td><td>Leu</td><td>Pro</td><td>Leu</td><td>Gly</td><td>-</td><td>Val</td><td>Leu</td><td>Leu</td><td>Leu</td><td></td><td></td></t<>	Ile 305	Val	Pro	Leu	Gln	-	Leu	Pro	Leu	Gly	-	Val	Leu	Leu	Leu		
340 345 350 Gly lle lle Gin Lys Asp Trp Ala Ala Met Gin Val Glu Thr Thr Gin 355 360 365 Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu 370 380 380 Asp Tyr Gin Ala Val His His Leu Phe Pro Asm Val Ser Gin His His 400 400 Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asm Thr Cys Ser Glu Tyr Lys 405 415 Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gin Ala Phe Ala Ser His 430 425 Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu 445 445 C210> SEQ ID NO 15 421 C211> LENGINSM: Cencontabditis elegans 445 C220> FEATURE: 422 C210> SEQ UENCE: 15 425 atg gt atta Gga gag Caa gag Cat gag cat gag cat dag at C act act act ag gat Gal gag for Gly 10° 48 Met Val Leu Arg Glu Gln Glu His Glu Pro Phe Phe Ile Lyg Ile Asp 10° 96 Gly Lys Tr Cys Gln Ile Asp Asp Asp Asp An Met Asp Ala Thr Nv 1 Phe 30° 40 10 15 96 Gly Lys Tr Cys Glu Ile Asp Asp Asp Asp Met Asp Ala Thr Thr Val Phe 45 41 10 16 110 110 11 110 115 110 221<>NAME/MEY: CBS 29 110 15 96 <td>Thr</td> <td>Val</td> <td>Ala</td> <td>Asp</td> <td></td> <td>Val</td> <td>Ser</td> <td>Ser</td> <td>Tyr</td> <td>_</td> <td>Leu</td> <td>Ala</td> <td>Leu</td> <td>Thr</td> <td></td> <td>Gln</td> <td></td>	Thr	Val	Ala	Asp		Val	Ser	Ser	Tyr	_	Leu	Ala	Leu	Thr		Gln	
355 360 365 Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu 380 370 All His His Leu Phe Pro Asm Val Ser Gln His His 390 390 Ser Glu Tyr Lys 400 400 Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys 405 410 Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His 420 425 440 445 421 440 425 440 445 420 440 445 421 440 422 440 445 421 Leu Gly Leu Arg Pro Lys Glu Glu Glu 445 4221 NAME/KEY: CDS 4222 CATTON: (1) (1344) 4222 CATTON: (1) (1344) 4222 CATTON: (1) (1344) 4222 CATTON: (1) (1344) 4222 Asg dgg caa gag cat gag cat gag cca tac cat cac cag ga 96 10 15 16 11 So 10 15 320 Glu Glu Glu His Glu Pro Phe Phe Ile	Ala	Asn	His		Val	Glu	Glu	Val		Trp	Pro	Leu	Pro	-	Glu	Asn	
370375380Aen Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His 395395400Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys 405410Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His 420425Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His 420435Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu 435446*210> SEQ ID NO 15 *211> LENGTH: 1344 *212> TYPE: DNA *212> CACATION: Concordabilis elegans *222> DECATION: 10:(1344) *222> LOCATION: CD: *15*400> SEQUENCE: 15486*400> SEQUENCE: 1510*41 Leu Arg Glu Gln Glu His Glu Pro Phe Phe Ile Lys Ile Asp 11151015*21 NAME/KEY: CDS *222> OCATION: 10: LeiA-5 desaturase*400> SEQUENCE: 15atg gta tta cga gag caa gag cat gag cca ttc ttc att aaa att gat 1048Met Val Leu Arg Glu Gln Glu His Glu Pro Phe Phe Ile Lys Ile Asp 1015*101530*2025*21SC Gli Ile Asp Asp Ala Val Leu Arg Ser His Pro Gly 2030*2021*2025*21*20*21*20*21*20*21*20*21*20*21*20*22*21*22*21*22*21*22*21*22*21*22*21*21*24*21*26*23*27*24*26 <td>Gly</td> <td>Ile</td> <td></td> <td>Gln</td> <td>ГЛа</td> <td>Aap</td> <td>Trp</td> <td></td> <td>Ala</td> <td>Met</td> <td>Gln</td> <td>Val</td> <td></td> <td>Thr</td> <td>Thr</td> <td>Gln</td> <td></td>	Gly	Ile		Gln	ГЛа	Aap	Trp		Ala	Met	Gln	Val		Thr	Thr	Gln	
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405 410 415 Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His 420 420 425 430 420 425 420 Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu 445 435 440 445 421> TYPE: DNA 440 421> TYPE: DNA 422> DOCATION: (1)(1344) 422> LOCATION: (1)(1344) 422> LOCATION: 1)(1344) 422> DOCATION: 1)(1344) 422> DOCATION: 1) 10 10 15 gag aaa tgg tgt caa att gac gat gct gtc ttc aat aa att gat Mak Val Leu Arg Ser His Pro Gly 40 20 20 25 30 20 21 And Ala Phe Ala Val Leu Arg Ser His Pro Gly 32 40 45 <td< td=""><td>Asn 385</td><td>Tyr</td><td>Gln</td><td>Ala</td><td>Val</td><td></td><td>His</td><td>Leu</td><td>Phe</td><td>Pro</td><td></td><td>Val</td><td>Ser</td><td>Gln</td><td>His</td><td></td><td></td></td<>	Asn 385	Tyr	Gln	Ala	Val		His	Leu	Phe	Pro		Val	Ser	Gln	His		
420425430Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu 435440445435440445440445 <td>Tyr</td> <td>Pro</td> <td>Asp</td> <td>Ile</td> <td></td> <td>Ala</td> <td>Ile</td> <td>Ile</td> <td>Lys</td> <td></td> <td>Thr</td> <td>Суз</td> <td>Ser</td> <td>Glu</td> <td>-</td> <td>Lys</td> <td></td>	Tyr	Pro	Asp	Ile		Ala	Ile	Ile	Lys		Thr	Суз	Ser	Glu	-	Lys	
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MetValLeuArgGluGluGluHisGluProPhePheIleLysIleAsp111 <td< th=""><th><221 <222 <223</th><th>L> NA 2> LC 3> OT</th><th>AME/H DCATI THER</th><th>CEY : ION : INF(</th><th>(1) ORMA</th><th></th><th></th><th>lta-5</th><th>5 de:</th><th>satu</th><th>case</th><th></th><th></th><th></th><th></th><th></th><th></th></td<>	<221 <222 <223	L> NA 2> LC 3> OT	AME/H DCATI THER	CEY : ION : INF((1) ORMA			lta-5	5 de:	satu	case						
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GIY Lys Trp Cys Gln Ile Asp Asp Ala Val Leu Arg Ser His Pro Gly ggt agt gca att act act act tt aaa aat atg gat gcc act acc gta ttc 144 Gly Ser Ala Ile Thr Thr Tyr Lys Asn Met Asp Ala Thr Thr Val Phe ala att act cat act gtt tt aaa aat atg gcc act acc gaa cat caa gaa cca gaa cca gaa acc gaa aca gaa fla fla gaa cca gaa cca gaa acc gaa act caa gaa cca gaa fla fla gaa fla	1			0	5					10				-	15	-	
Gly Ser Ala Ile Thr Thr Tyr Lys Asn Met Asp Ala Thr Thr Val Phe 35 Image: Assn Met Asp Ala Thr Thr Val Phe 40 Image: Assn Met Asp Ala Thr Thr Val Phe 45 cac aca ttc cat act ggt tct aaa gaa gcg tat caa tgg ctg aca gaa His Thr Phe His Thr Gly Ser Lys Glu Ala Tyr Gln Trp Leu Thr Glu 50 192 ttg aaa aaa gag tgc cct aca caa gaa cca gag atc cca gat att aag Leu Lys Lys Glu Cys Pro Thr Gln Glu Pro Glu Ile Pro Asp Ile Lys 65 240 gat gac cca atc aaa gga att gat gat gtg aac atg gga act ttc aat 85 288 Asp Asp Pro Ile Lys Gly Ile Asp Asp Val Asn Met Gly Thr Phe Asn 90 95 att tct gag aaa cga tct gcc caa ata aat aat aaa agt ttc act gat cta 100 336 cgt atg cga gtt cgt gca gaa gga ctt atg gat gga tgg tgg tct cct ttg ttc 100 384				Cys			Asp	Asp	Ala								96
His Thr Phe His Thr Gly Ser Lys Glu Ala Tyr Gln Trp Leu Thr Glu 50 55 60 70 60 ttg aaa aaa gag tgc cct aca caa gaa cca gag atc cca gat att aag 240 240 Leu Lys Lys Glu Cys Pro Thr Gln Glu Pro Glu Ile Pro Asp Ile Lys 80 240 65 70 75 80 288 gat gac cca atc aaa gga att gat gat gtg aac atg gga act ttc aat 288 288 Asp Asp Pro Ile Lys Gly Ile Asp Asp Val Asn Met Gly Thr Phe Asn 90 95 att tct gag aaa cga tct gcc caa ata aat aat aat agt ttc act gat cta 100 336 Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp Leu 100 105 384 cgt atg cga gtt cgt gca gaa gga ctt atg gat gga tct cct ttg ttc 384 Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe 384			Āla					Lys					Thr				144
Leu Lys Lys Glu Cys Pro Thr Gln Glu Pro Glu Ile Pro Asp Ile Lys 65 70 75 80 gat gac cca atc aaa gga att gat gat gtg aac atg gga act ttc aat 288 Asp Asp Pro Ile Lys Gly Ile Asp Asp Val Asn Met Gly Thr Phe Asn 85 90 95 att tct gag aaa cga tct gcc caa ata aat aaa agt ttc act gat cta 336 Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp Leu 100 105 110 110 110 384 Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe		Thr					Ser					Gln					192
Asp Asp Pro Ile Lys GIy Ile Asp Asp Val Asn Met GIy Thr Phe Asn 90 95 att tct gag aaa cga tct gcc caa ata aat aaa agt ttc act gat cta 336 Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp Leu 100 105 cgt atg cga gtt cgt gca gaa gga ctt atg gat gga tct cct ttg ttc 384 Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe 384					-	Pro			-		Glu			-		Lys	240
Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp Leu 100 105 110 cgt atg cga gtt cgt gca gaa gga ctt atg gat gga tct cct ttg ttc 384 Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe	-	-			Lys			-	-	Val		-			Phe		288
Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe				Lys	-		-		Ile			-		Thr	-		336
	-	-	Arg	-	-	-	-	Gly		-	-		Ser		-		384

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	att Ile 130															432
	ctt Leu										-			-		480
	gcg Ala															528
	ttg Leu															576
	aac Asn															624
	gtg Val 210															672
	tta Leu															720
	cag Gln	-		~~	-	-				-				-		768
	aca Thr		-					-		-				-		816
	att Ile															864
	act Thr 290															912
	ttg Leu			-					-				-		-	960
	ttc Phe															1008
	act Thr						Val									1056
	atg Met				-	-				-			-		-	1104
	cct Pro 370															1152
	gag Glu						_	_		-			-			1200
	atg Met															1248
	gtc Val															1296
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Gly	Lys	Trp	Cys 20	Gln	Ile	Asp	Asp	Ala 25	Val	Leu	Arg	Ser	His 30	Pro	Gly
Gly	Ser	Ala 35	Ile	Thr	Thr	Tyr	Lys 40	Asn	Met	Asp	Ala	Thr 45	Thr	Val	Phe
His	Thr 50	Phe	His	Thr	Gly	Ser 55	Lys	Glu	Ala	Tyr	Gln 60	Trp	Leu	Thr	Glu
Leu 65	Lys	Lys	Glu	Сүз	Pro 70	Thr	Gln	Glu	Pro	Glu 75	Ile	Pro	Asp	Ile	Lys 80
Asp	Asp	Pro	Ile	Lys 85	Gly	Ile	Asp	Asp	Val 90	Asn	Met	Gly	Thr	Phe 95	Asn
Ile	Ser	Glu	Lys 100	Arg	Ser	Ala	Gln	Ile 105	Asn	Lys	Ser	Phe	Thr 110	Aab	Leu
Arg	Met	Arg 115	Val	Arg	Ala	Glu	Gly 120	Leu	Met	Asp	Gly	Ser 125	Pro	Leu	Phe
Tyr	Ile 130	Arg	Lys	Ile	Leu	Glu 135		Ile	Phe	Thr	Ile 140		Phe	Ala	Phe
Tyr 145			Tyr	His	Thr 150		Tyr	Leu	Pro	Ser 155		Ile	Leu	Met	Gly 160
	Ala	Trp	Gln	Gln 165		Gly	Trp	Leu	Ile 170		Glu	Phe	Ala	His 175	
Gln	Leu	Phe	Lys 180		Arg		Tyr	Asn 185		Leu	Ala	Ser	Tyr 190		Val
Gly	Asn	Phe 195	Leu	Gln			Ser 200		Gly	-		Lys 205		Gln	His
Asn	Val 210	His	His	Ala	Ala	Thr 215		Val					Gly	Asp	Leu
Asp 225			Pro	Phe	Tyr 230	Ala	Thr	Val	Ala	Glu 235		Leu	Asn	Asn	Tyr 240
	Gln	Asp	Ser	Trp 245			Thr	Leu	Phe 250		Trp	Gln	His	Val 255	
Trp	Thr	Phe	Met 260		Pro	Phe	Leu	Arg 265		Ser	Trp	Leu	Leu 270		Ser
Ile	Ile	Phe 275	Val	Ser	Gln	Met	Pro 280		His	Tyr	Tyr	Asp 285		Tyr	Arg
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Ser 305			Gln	Leu	Tyr 310		Leu	Pro	Asp	Trp 315		Thr	Arg	Ile	Met 320
	Phe	Leu	Val			Leu	Val	Gly			Leu	Leu	Ser		
Val	Thr	Phe	Asn	325 His	Tyr	Ser	Val		730 730	Phe	Ala	Leu		335 Ser	Asn
Ile	Met	Ser	340 Asn	Tyr	Ala	Cys	Leu	345 Gln	Ile	Met	Thr	Thr	350 Arg	Asn	Met
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Arg	Pro 370	Gly	Arg	Phe	Ile	Asp 375	Trp	Leu	Trp	Gly	Gly 380	Leu	Asn	Tyr	Gln	
Ile 385	Glu	His	His	Leu	Phe 390	Pro	Thr	Met	Pro	Arg 395	His	Asn	Leu	Asn	Thr 400	
Val	Met	Pro	Leu	Val 405	Гла	Glu	Phe	Ala	Ala 410	Ala	Asn	Gly	Leu	Pro 415	Tyr	
Met	Val	Asp	Asp 420	Tyr	Phe	Thr	Gly	Phe 425	Trp	Leu	Glu	Ile	Glu 430	Gln	Phe	
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		-	NCE: ccct		aa g	agagi	tagt	c ati	ttt	catc			-		a atc	56
											1				ln Ile 5	
												gat Asp				104
-				-					-		-	gtt Val	-	-		152
		-					-			-	-	agt Ser 50		-		200
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												aaa Lys				296
-			-			-			-			ttt Phe				344
	-		-		-						-	ttt Phe	-		-	392
												999 Gly 130				440
												ttg Leu				488
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	-					-						tgg Trp				632

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His His Ile Åla Cýs Asn Šer Leu Ĝlu Tyr Åsp Pro Åsp Leu Gln 200 205 201 205 202 200 202 200 203 205 204 200 205 200 206 200 207 200 208 200 200 200 201 200 202 200 201 200 201 200 201 200 201 200 202 200 201 200 202 200 203 201 204 205 205 200 206 200 207 201 208 77 201 202 202 200 203 201 204 202 205 200 206 200 207 201 208 77														0 1 1 1			
Ile pro Phe Leu Val Val Ser Ser Lys Phe Phe Gly Ser Leu Thr 215 220 cat ttc tat gag aaa agg ttg act ttt gac tct tta tca agg ttc 776 His Phe Tyr Glu Lys Arg Leu Thr Phe App Ser Leu Ser Arg Phe 245 gta agt tat caa cat tgg aca ttt tac cat att atg tgg tgg cg gct 824 Val Ser Tyr Gln His Tp Thr Phe Tyr Pro Ile Met Cys Ala Ala 250 zta tat gt at gtg caa tct ctc att atg ttg tga ca agg ag 872 Leu Asm Met Tyr Val Gln Ser Leu Ile Met Leu Uan Try Ay Arg 270 zta tat ct cg ag tc ag ga act cttg gg tg cd ag tg tct tg 920 val Ser Tyr Arg Ala Gln Glu Leu Leu Gly Cys Leu Val Phe Ser 285 zta cog ttg ctt gtt ctt tgt ttg cat at tgg ggt gaa aga 960 Thp Tyr Pro Leu Leu Val Ser Cys Leu Pro Am Trp Gly Glu Arg 325 atg ttt gtt att gca agt tta tog gtg atg caa caa gtt 1016 Met Phe Val Ile Ala Ser Leu Ser Val Thr Gly Met Gln Gln Val 325 atg ttt gt att gg aga cac tc gg at tg gt ga at att gg tg ga tg ga aag cct 1064 Phe Ser Leu Aan His Phe Ser Ser Ser Val Tyr Val Gly Lys Pro 330 330 335 326 345 gg ag at att tg gt tg gt tg gt tg gt ga tg ga act ga act ta gad att 1112 Gly Ann Arr Phe Glu Lys Gln Thr App Gly Thr Leu App Ile <t< td=""><td></td><td></td><td>His</td><td></td><td></td><td></td><td></td><td>Ser</td><td></td><td></td><td></td><td></td><td>Pro</td><td></td><td></td><td></td><td>680</td></t<>			His					Ser					Pro				680
His Phe Tyr Glu Lys Arg Leu Thr Phe Asp Ser Leu Ser Arg Phe 235245gta agt tat caa cat tgg aca ttt tac oct att atg tgt get got 250824val Ser Tyr Gln His Trp Thr Phe 250Trp Thr Phe 255777Leu Aan Met Tyr Val Gln Ser Leu Ile Met Leu Lue Thr Lys Arg 265827Leu Aan Met Tyr Val Gln Ser Leu Ile Met Leu Lue Thr Lys Arg 265920yal Ser Tyr Arg Ala Gln Glu Leu Leu Gly Cys Leu Val Phe Ser 280920tgg tac ccg ttg ctt gtt tct tgt ttg cct aat tgg ggt gaa aga 295900tgg tac ccg ttg ctt gtt tct tgt ttg cct aat tgg ggt gaa aga 295900atg ttt gtt att goa agt tta tca gtg act gga atg caa caa gtt 3151016Met Phe Val Ile Ala Ser Leu Ser Val Thr Gly Met Gln Gln Val 310325ttc tcc ttg acc act tct ct tc ta agt ggt agg acc tt gaa aga 3101064Phe Ser Leu Asn His Phe Ser Ser Ser Val Tyr Val Gly Lys Pro 33010164Gly Aan Ann Trp Phe Glu Lys Gln Thr App Gly Thr Leu Asp Ile 3461112Gly Aan Ann Trp Phe Glu Lys Gln Thr App Gly Lue Gln Phe Gln 365375gg cat cat ttg ttt ccc aag tg ct aga tgc aac ctt agg aaa 3671064yas cat cat ttg tt ccc aag agg ccc aga aca ctt agg aaa 3681206ccg ccc tac gtg at ggat tag tat tcg aag aca ct aga tgc aat tt 4001208gg cat cat ttg tt cca aga gtg ccc aga aga cat aat ttg ctt ac 375336tg cat cat ttg tt cca aga gtg cat aga aca cat agg aaa 3761206gg at aat tgg at gga ta ac aga gtg cac act aat ttg ct tac 360315ttg ccc ct cct gg ag tg aga tta tgc aag aca ct aga acat tag 376326<		Ile					Val					Phe					728
ValSerTyr Gln His Trp ThrPheTyr ProIle MetCys Ala Åla Åla255255255270275260ctoaat atg tat gta caa tot oto ata atg ttg ttg acc aag aga 265270275275gtg toc tat oga got cag gaa oto ttg gga tgo cta gtg tto tog 285270275270gtg toc tat oga got cag gaa oto ttg gga tgo cta gtg tto tog 285290290290tgg tac cog ttg ott gtt tot tgt ttg ttg cot aat tgg ggt gaa aga 295300305305atg ttt gtt att gca agt tta tog gg atg cag agg caa caa ggt 315300305325tto toc ttg aac cac tto tot to a gg gt gg aag gct 33010161016Met Phe Val Ile Ala Ser Leu Ser Val Thr Gly Met Gln Gln Val 3153251016gg aat aat tgg ttt ga aaa caa acg gat gg ga ca ctt gac att 3303401112gg aat aat tgg ttt gag aaa caa acg gat gg ga at dto aab ap Trp Phe 330370370340gg act cat ttg ttt cc aag atg ot agg tg gaa ttg caa tto caa 365370370370gg ag ct cat ttg ttt cc aag atg ot agg tg cac ctt agg aaa 3663703851066gag cat cat ttg ttt cc aag atg caa caa at att tg ct ag 400120812081208gtu the Ibe UP Pro 3953902853851208gt ctt tto toc aag gc agg cat at aga aag aca ct aga aca ttg 375380365370ga ct at ttg tth cc aag gc aat gaa atg aca ct aga aca ttg 39513041208gtu the Gu Leu Cys Lys Lys His Asn Leu Pro 39512081		His				Lys		-			Āsp				-	Phe	776
Leu Asn Met Tyr Val Gln Ser Leu IIe Met Leu Lu Thr Lys Arg 265 Tyr Arg Ala Gln Glu Leu Leu Gly Cys Leu Val Phe Ser 280 Tyr Tyr Arg Ala Gln Glu Leu Leu Gly Cys Leu Val Phe Ser 280 Tyr Tyr Pro Leu Leu Val Ser Cys Leu Pro Asn Trp Gly Glu Arg 305 305 tgg tac ccg ttg ctt gtt tct tgt ttg cct aat tgg ggt gaa aga 4968 Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro Asn Trp Gly Glu Arg 305 atg ttt gtt att gca agt tta tca gtg atg gaa ag cca caa gtt 1016 Met Phe Val IIe Ala Ser Leu Ser Val Thr Gly Met Gln Gln Val 315 320 325 ttc tcc ttg aac cac ttc tct tca agt gtt tat gtt gga aag cct Phe Ser Leu Asn His Phe Ser Ser Val Tyr Val Gly Lys Pro 330 335 tg cct cct tgg atg gat tg tt cat ggt gga ca ctt gac att Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp Gly Thr Leu Asp IIe 346 355 tg cct cct tgg atg gat tg tt cat ggt gga ttg caa ttc caa Cys Pro Pro Trp Met Asp Trp Phe His Gly Gly Leu Gln Phe Gln 366 370 360 370 362 tag cac tat ttg tt ccc aag atg cct aga tgc caa ctt agg asa Glu His His Leu Phe Pro Lys Met Pro Arg Cys Asn Leu Arg Lys 375 380 tag cac cat ttg ccc aag gcc aat gaa atg aca ct aga acat ttg 400 405 tat gca tct ttc tcc aag gcc aat gaa atg aca ct aga acat ttg 410 410 415 410 415 420 415 tat gca tct ttc tcc aag gcg atg gaa tg aca ct aga acat ttg 420 435 tat gca tct ttc tcc aag gcc aat gaa atg aca ct aga acat ttg 430 435 tat gca tct ttc tcc aag gcc aat gaa atg aca ct aga acat ttg 435 tat gca tct ttc tcc aag gcc aat gaa atg aca ct aga acat ttg 435 tat gca tct ttc tcc aag gcc aat gaa atg aca ct aga acat ttg 435 tat gca tct ttc tcc aag gcc aat gaa atg aca ct aga acat ttg 440 445 tat gca tct ttc tcc aag gcc att gag tat acc aag ccg ct c ccg aag 435 tag gta tgg gaa gct ctt cca act cat ggt taa aattaccctt 436 teu Val Trp Glu Ala Leu His Thr His Gly 440 445 taggagt cattgcaact tgtctttat ggttattag atgttttta atatattta 456 taggagt cattgcaact tgtctttat ggttattag atgttttta atatattta 457 458 459 459 450 459 450 459 450 450 450 450 450 450 450 450					Gln					Tyr					Āla		824
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Phe Ser Leu Asn His Phe Ser Ser Ser Val Tyr Val Gly Lys Pro 330340ggg aat aat tgg ttt gag aaa caa acg gat ggg aca ctt gac att Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp Gly Thr Leu Asp Ile 3451112gt cct cct tgg atg gat tgg ttt cat ggt gga ttg caa ttc caa Cys Pro Pro Trp Met Asp Trp Phe His Gly Gly Leu Gln Phe Gln 3651160gag cat cat ttg ttt ccc aag atg cct aga tgc aac ctt agg aaa Glu His His Leu Phe Pro Lys Met Pro Arg Cys Asn Leu Arg Lys 3751208tcg ccc tac gtg atc gag tta tgc aat Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys His Asn Leu Pro Tyr 4001256tat gca tct ttc tcc aag gcc aat gaa atg aca ctc aga aca ttg Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met Thr Leu Arg Thr Leu 4101304aac aca gca ttg cag gct atg cac act cat ggt taa aattaccctt 4251398ttg gta tgg gaa gct ctt cac act cat ggt taa aattaccctt 4401398ttg gta tgg gaa gt ttgttatta ggtttattag atgttttta atatttta1518gt ttggag cattgcaact tgtctttat 4401518ttggag tatattgg aataaggagt tgcatattgt caattgt 1578		Met				Āla					Thr					Val	1016
Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp Gly Thr Leu Asp Ile 345350355tgt cct cct tgg atg gat tgg ttt cat ggt gga ttg caa ttc caa Cys Pro Pro Trp Met Asp Trp Phe His Gly Gly Leu Gln Phe Gln 3601160gag cat cat ttg ttt ccc aag atg cct aga tgc aac ctt agg aaa Glu His His Leu Phe Pro Lys Met Pro Arg Cys Asn Leu Arg Lys 3751208tcg ccc tac gtg atc gag tta tgc aag aaa Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys His Asn Leu Pro Tyr 3951256tcg ccc tac gtg atc gag tta tgc aag aaa ser Pro Tyr Val Ile Glu Leu Cys Lys Lys His Asn Leu Pro Tyr 4001304tat gca tct ttc tcc aag gcc aat gaa atg aca ctc aga aca ttg 4101304Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met Thr Leu Arg Thr Leu 410415aac aca gca ttg cag gct agg gat ata acc 425aag cac ccc gaag 430ttg gta tgg gaa gct ctt cac 4451398ttg gta tgg gaa gct ctt cac act cat ggt taa 4451398tcatgta ataatttgag attatgtatc tcctatgttt 4451398tcatgta ataatttgag attatgtatc tcctatgttt ggtttgc tttcatctcc attattgatg aataaggagt tggattatgt cattgcaact tgtctttat 4451518ttggagt cattgcaact tgtcttttat ggtttattag atgttttta attatttta 15181518ttggagt cttcaccctc attattgatg aataaggagt tgcatattgt caattgt caattgttg tcaatgta1578	-			-	Asn					Ser	-		-		Lys		1064
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Glu His His Leu Phe Pro Lys Met Pro Arg Cys Asn Leu Arg Lys 380385tcg ccc tac gtg atc gag tta tgc aag aaa cat aat ttg cct tac Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys His Asn Leu Pro Tyr 3951256tat gca tct ttc tcc aag gcc aat gaa atg aca ctc aga aca ttg Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met Thr Leu Arg Thr Leu 4101304aac aca gca ttg cag gct agg gat ata acc aag ccg ctc ccg aag Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr Lys Pro Leu Pro Lys 4251352ttg gta tgg gaa gct ctt cac act cat ggt taa aattaccctt Leu Val Trp Glu Ala Leu His Thr His Gly 4401398ttggagt cattgcaact tgtcttttat ggtttattag atgttttta atatttta1518ttggagt cattgcaact tgtcttttat ggtttattag atgttttta atatttta1518gttttgc tttcatctcc attattgatg aataaggagt tgcatattgt caattgttgt1578tat ggaattttg gaatgtactt tgtaccactg tgttttcagt tgaagctcat1638			Pro					Trp					Leu				1160
Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys His Asn Leu Pro Tyr 395 400 405 tat gca tct ttc tcc aag gcc aat gaa atg aca ctc aga aca ttg Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met Thr Leu Arg Thr Leu 410 1304 aac aca gca ttg cag gct agg gat ata acc aag ccg ctc ccg aag Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr Lys Pro Leu Pro Lys 425 1352 ttg gta tgg gaa gct ctt cac act cat ggt taa aattaccctt 1398 Leu Val Trp Glu Ala Leu His Thr His Gly 440 445 ttggagt cattgcaact tgtcttttat ggtttattag atgttttta atatattta 1518 gtttgc tttcatctcc attattgatg aataaggagt tgcatattgt caattggtgt 1578 gttttgc tttcatctcc attattgatg attatgatct tgtaccactg tgttttcagt tgaagctcat 1638		Glu					Pro					Cys					1208
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Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr Lys Pro Leu Pro Lys 425 430 435 ttg gta tgg gaa gct ctt cac act cat ggt taa aattaccctt 1398 Leu Val Trp Glu Ala Leu His Thr His Gly 440 445 tcatgta ataatttgag attatgtatc tcctatgttt gtgtcttgtc ttggttctac 1458 ttggagt cattgcaact tgtcttttat ggtttattag atgttttta atatattta 1518 gttttgc tttcatctcc attattgatg aataaggagt tgcatattgt caattgttgt 1578 caatatc tgatatttg gaatgtactt tgtaccactg tgttttcagt tgaagctcat 1638					Phe					Glu					Thr		1304
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	cag Gln															1344
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Leu	Glu	Leu	Leu 180	ГЛа	Glu	Tyr	Arg	Glu 185	Leu	Arg	Ala	Leu	Phe 190	Leu	Arg
Glu	Gln	Leu 195	Phe	ГЛа	Ser	Ser	Lys 200	Ser	Tyr	Tyr	Leu	Phe 205	Lys	Thr	Leu
Ile	Asn 210	Val	Ser	Ile	Val	Ala 215	Thr	Ser	Ile	Ala	Ile 220	Ile	Ser	Leu	Tyr
Lys 225	Ser	Tyr	Arg	Ala	Val 230	Leu	Leu	Ser	Ala	Ser 235	Leu	Met	Gly	Leu	Phe 240
Ile	Gln	Gln	Суз	Gly 245	Trp	Leu	Ser	His	Asp 250	Phe	Leu	His	His	Gln 255	Val
Phe	Glu	Thr	Arg 260	Trp	Leu	Asn	Asp	Val 265	Val	Gly	Tyr	Val	Val 270	Gly	Asn
Val	Val	Leu 275	Gly	Phe	Ser	Val	Ser 280	Trp	Trp	Гла	Thr	Lys 285	His	Asn	Leu
His	His 290	Ala	Ala	Pro	Asn	Glu 295	Суз	Asp	Gln	Lys	Tyr 300	Thr	Pro	Ile	Asp
Glu 305	Asp	Ile	Asp	Thr	Leu 310	Pro	Ile	Ile	Ala	Trp 315	Ser	ГЛЗ	Asp	Leu	Leu 320
Ala	Thr	Val	Glu	Ser 325	Lys	Thr	Met	Leu	Arg 330	Val	Leu	Gln	Tyr	Gln 335	His
Leu	Phe	Phe	Leu 340	Val	Leu	Leu	Thr	Phe 345	Ala	Arg	Ala	Ser	Trp 350	Leu	Phe
Trp	Ser	Ala 355	Ala	Phe	Thr	Leu	Arg 360	Pro	Glu	Leu	Thr	Leu 365	Gly	Glu	LYa
Leu	Leu 370	Glu	Arg	Gly	Thr	Met 375	Ala	Leu	His	Tyr	Ile 380	Trp	Phe	Asn	Ser
Val 385	Ala	Phe	Tyr	Leu	Leu 390	Pro	Gly	Trp	Lys	Pro 395	Val	Val	Trp	Met	Val 400
Val	Ser	Glu	Leu	Met 405	Ser	Gly	Phe	Leu	Leu 410	Gly	Tyr	Val	Phe	Val 415	Leu

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Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp 2he Val Asn 420 Ala Gh I Ha La Ser Thr Arg Asp I he Lys Ala Gly Val Phe Ann Asp 425 Thr Phe Thr Gly Gly Leu Asn Arg Gln I he Glu His His Leu Phe Pro 450 Thr Met Pro Arg His Ann Leu Ann Lys I he ser Pro His Val Glu Thr 465 Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ser Met Ala Ser 485 Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ash Asp Ala Ala Ser 510 Ser His Gln Gin Leu Ala Ala Ser 520 <pre>clin Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ala Asp Ala Ala Ser 485 Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala Ser 510 Ser His Gln Gin Leu Ala Ala Ser 520 </pre>																	
435440445Trp Phe Thr Gly Gly Leu Asn Arg Gln He Glu Hie His Leu Phe Pro 455455Thr Met Pro Arg His Asn Leu Asn Lys IIe Ser Pro His Val Glu Thr 470470445470475Leu Cys Lys Lys Hie Gly Leu Val Tyr Glu App Val Ser Met Ala Ser 490495Gly Thr Tyr Arg Val Leu Lyo Thr Leu Lys Asp Val Ala Apr Ala Ala 505Ser His Gln Gln Leu Ala Ala Ser 510-2110 - SEQ ID NO 21 -2112 TYPE: DNA -2120 TYPE: DNA -2130 CGATION: (1) (1434) -2120 TYPE: DNA -2120 TYPE: DNA -210 TS4200 SEQUENCE: 21 at gg gc aaa gga ggg gac gct cgg gcc tcg aag gc tca acg gcg gct 48 1 510 15Gc aag at c agt tog cag gaa gtc aag acc acg cg tc tcg ade gg ac 20 Typ Glu Glu Val Lyp And Arg Ala Ser Lyp Gly Ser Thr Ala Ala 1 511 15Gc tag at c act tog ga gad tc aag acc acg cg tc tca act gg acg act 20 Typ Glu Glu Val Lyp And Ala Typ Ang Val Ser Ann Typ His 3512330341345345346347348349349341341345345346347348348349349349341341345346<	Ser	His	Asn	-	Met	Glu	Val	Tyr		Thr	Ser	Lys	Asp		Val	Asn	
450 455 460 Thr Met Pro Arg His Am Leu An Lyu Ile Ser Pro His Val Glu Thr 455 470 470 470 Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser 495 475 475 Gly Thr Tyr Arg Val Leu Lys Thr Lau Lys Amp Val Ala Ang Ala Ala 500 501 501 Ser His Gln Gln Leu Ala Ala Ser 515 520 -2210 SEQ ID NO 21 -2213 ORGANISM: Phaeodactylum tricornutum -2213 ORGANISM: Phaeodactylum tricornutum -2210 PEATURE: -2223 OTHER INFORMATION: Delta-5 desaturase -2210 SEQUENCE: 21 314 atg gge aaa gga ggdg ac gct cgg gcc tcg aag ggc tca acg gcg gct 480 48 1 5 10 15 gcc tgg ac att cgg cg gaa gac cas ag cc cas gcg ct cc ga ga gac Arg Lys ILe Ser Thr Glu Val Lys Thr His Ala Ser Pro Glu Aap 20 144 1 5 5 30 gcc tgg ac att cag tcc aat ag gt ta gac cac gca gt gc cas at gg ca at gg 20 144 41 5 10 144 35 5 5 10 gcc tgg ac att cac tcc aat aag gt tac gac cac geg tcg cas at gg ca at g 20 20 20 ga cat ccc gga gge gcc gt dt tt cac gca gca cac geg tcg cat at g 20 30 192 ag aga cat tcc gc tt tc aat tag gc tac cac geg at ca dt cg c	Ala	Gln		Ala	Ser	Thr	Arg		Ile	Lys	Ala	Gly		Phe	Asn	Asp	
465 470 475 480 Leu Cys Lys Lys Hig Gly Leu Val Tyr Glu App Val Ser Met Ala Ser 485 500 Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys App Val Ala App Ala Ala 500 Ser Hig Gln Gln Leu Ala Ala Ser 500 Ser Hig Gln Gln Leu Ala Ala Ser 500 500 *210> SEQ ID NO 21 500 *211> LENNTH: 1434 500 *212> TOFER: DNA 4213> ORGANISM: Phaeodactylum tricornutum *222> EPATTORE: 480 *222> LOCATOM: (1) (1434) *223> OTHER INFORMATION: Delta-6 desaturase *400> SEQUENCE: 21 Atg gge aad gga gge get cegg get ceg and geg eff ce aad geg gg tha Ala 10 15 5 200 REATION: (1) (1434) *223> COTHER: INFORMATION: Delta-6 desaturase *400> SEQUENCE: 21 Atg gge aad gat agt cag ag ang te ang ce cae ged to t ceg ang ge ta ang ge ge ta and ta ge ang gat ang ta ge ang gat ge ta ge ge get get get get get get get get g	Trp		Thr	Gly	Gly	Leu		Arg	Gln	Ile	Glu		His	Leu	Phe	Pro	
Autor 485490495Gly Thr Tyr Arg Val Lew Lye Thr Lew Lye Aep Val Ala Aep Ala Ala 500Ser His Gln Gln Lew Ala Ala Ser 515<210> SEQ ID NO 21 <1115 LENGTH: 1434		Met	Pro	Arg	His		Leu	Asn	Lys	Ile		Pro	His	Val	Glu		
See His Gin Gin Leu Ala Ala Ser 515 50 1 500 1 See His Gin Gin Leu Ala Ala Ser 515 520 		-	-	-	485	-			-	490	_				495		
 515 520 210 SEQ ID NO 21 2113 LENGTH: 1434 2123 FYER DNA 2123 SIMME/KET: CDS 2223 NAME/KET: CDS 2223 OTHER INFORMATION: Delta-6 desaturase 2233 OTHER INFORMATION: Delta-6 desaturase 2233 OTHER INFORMATION: Delta-6 desaturase 2230 Grad ggg ggg ggg gg cd cg cg gg cc cg gg cc ca gg gg cc cg gg gg c 233 Grad gg ca ad ggg ggg gg ag gg ca cg cg ca ad gg gc tac ac gg gg tac ac gg gg gg ga gg 2400 SEQUENCE: 21 251 AMME/KET: TDS 101 Cl (134) 251 Cg ga ad ggg ggg gg ag gg ag gg ca ag gg ca ad gg gg ag gg ag (200 Gg gg ca ad gg gg ca ad gg gg ca ad gg gg ca ad gg gg ag gg ag (200 Gg gg ca ad gg gg	-		-	500						Lys	Asp	Val	Ala	-	Ala	Ala	
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111015cgc aag atc agt tgg cag gaa gtc aag acc cac gcg tct ccg gag gac gac gac gag gac gac gad tg gac gad gac gac gad gac gac gac gac gad gac gac gac gac acc tcc gag ag gc cc dca at aag gtc tac gac gtg tcc aac tgg cac gac atg gac gac att ttc acc gca gca gca gca gac att gac gcc gcd gad tg gac gac atg gac gac att gac gcc gcd gad tg gac gac atg gac gac att gac gac gac gcc gad tg gac gac atg gac gac att gac gcc gcd gad tg gcc gad tg gac gac atg gac gac atg gac gac att gac gcc gcd gcd gac acc gcc gad gac gac gac gac atg gac gac att gac gcc gcc gcd gad tg gac gac atg gac gac att gac gcc gcd gad tg gac gac acc gcc gad add gad gac gac gac gac gac atg gac gac att gac gac gcc gcd gad tg gac gac acc gac gac gac gac gac gac ga	atg	ggc	aaa	gga	aaa												48
Arg Lys Ile Sor Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp 20gcc tgg atc att cac tcc aat aag gtc tac gac gtg tcc aac tgg cac Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His 40144Ala Trp Ile Ile His Ser Asn Lys Val Ile Phe Thr His Ala Gly Asp Asp Met 50192gaa cat ccc gga ggc gcc gtc att ttc acg cac gcc ggt gac gac atg 50192gu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met 50192acg gac att ttc gct gcc ttc cac gca ccc gga tcg cag tcg tc atg 55240arg gac att ttc gct gcc ttt cac gca ccc gga ac acc gcg aag gag Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu 90288Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu 95336ccg cag caa atc gcc ttt gaa aag ggc tac gac cg gat ctg cgc tcc aaa 100336cct atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac 100336cct atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac 100336ctt atc tcg gac cgc ttc tgg gcc acc ccg gcc gcc gcc gtg gcc gcc gtc dta 110336ctt tac tcg gac atg tcc tgg gcc acc ctg gcc gcc gcc gcc dta gcc 110336ctt tac tcg gac cgc ttc tgg gta cac ctg gcc gcc gcc gcc dta gcc 110432leu Ile Met Met Gly Met Phe 13511013512013612013713613813513913614913514014014014014014114214314414514614	Met 1	Gly	ГЛВ	Gly	G1y 5	Aap	Ala	Arg	Ala		ГЛЗ	Gly	Ser	Thr		Ala	
Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His 35 36 37 38 39 39 30 30 30 30 30 30 30 30 30 30				Ser					Lys					Pro			96
Glu His Pro Gly Gly Ala Val IIe Phe Thr His Ala Gly Asp Asp Met50acg gac att ttc gct gcc ttt cac gca ccc gga tcg cag tcg ctc atg Thr Asp IIe Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met 70240aag aag ttc tac att ggc gaa ttg ctc ccg gaa acc acc ggc aag gag Lys Lys Phe Tyr IIe Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu 95288ccg cag caa atc gcc ttt gaa aag ggc tac cgc gat ctg cgc tcc aaa 85336ccg cag caa atc gcc ttt gaa aag ggc tac cgc gat ctg cgc tcc aaa 100336cct atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac 100384Leu IIe Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr 115384aag tgc ctc agc aac atg gcc att tgg gcc gcc gcc gcc tct gtc 130432Lys Cys Leu Ser Asn Met Ala IIe Trp Ala Ala Ala Cys Ala Leu Val 130432ttt tac tcg gac cgc ttc tgg gta cac ctg gcc agc gcc gcc gtc atg ctg 150480ftt tac tcg gac acg ttg ttg gtg ttg gca cac gac ttt ctg cac 150160gaa aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac 150528gaa aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac 150528gaa aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac 150528gaa aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac 160528			Ile					Lys					Ser				144
Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met 80aag aag ttc tac att ggc gaa ttg ctc ccg gaa acc acc ggc aag gag Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu 90288ccg cag caa atc gcc ttt gaa aag ggc tac cgc gat ctg cgc tcc aaa 100336Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys 105336ctc atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac 115384Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr 120384Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val 130432ttt tac tcg gac cgc ttc tgg gta cac ctg gcc agc gcc gcc gcc gtc atg ctg 150480ttt tac tcg gac cgc ttc tgg gta cac ctg gca cac gac ttt ctg 150480gga aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac 165528gga aca ttc ttt cag cag tcg gga tgg ttg tg gca cac gac ttt ctg cac 165528		His					Val					Āla					192
LysLysPheTyrIleGlyGluLeuLeuProGluThrGlyLysGlu909090909095336ccgcaaatcgcctttgaaaagggctaccgcgcctcaaaa336ProGlnGlnIleAlaPheGluLysGlyTyrArgAspLeuArgSerLys336ctcatcatgatgggcatgttcaagtccaaaaagtggttctacgtcaagLeuIleMetMetGlyMetPheLysSerAsnLysTrpPheTyr125aagtgcctcagcaacatggccgccgccgcctgt432LysCysLeuSerAsnMetAlaAlaCysAlaLeuVal130135TrAlaAlaAlaCysAlaLeuVal432LysCysLeuSerAsnMetAlaCysAlaLeuVal432LysCysLeuSerAsnMetAlaAlaCysAlaLeuValAla130135TrAlaAlaSerAlaValMetLeuAlaAla145150TrValAla <td< td=""><td>Thr</td><td></td><td></td><td></td><td></td><td>Āla</td><td></td><td></td><td></td><td></td><td>Gly</td><td></td><td></td><td></td><td></td><td>Met</td><td>240</td></td<>	Thr					Āla					Gly					Met	240
ProGlnGlnIleAlaPheGluLysGlyTyrArgAspLeuArgSerLysctcatcatgatgggcatgttcaagtccaacaagtggttctacgtctac384LeuIleMetMetGlyMetPheLysSerAsnLysTrpPheTyr125384aagtgcctcagcaacatggccatttgggccgcctgtgctctcgtc432LysCysLeuSerAsnMetAlaIleTrpAlaAlaAlaCysAlaLeuVal130ttttactcggaccgcttctgggcacacctggccgtcgtcatg145145					Ile					Pro					Lys		288
Leu Ile Met Met GIy Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr 115 IIS IIS IIS IIS Asn Lys Trp Phe Tyr Val Tyr 125 IIS IIS IIS IIS IIS IIS IIS IIS IIS II				Ile					Gly					Arg			336
Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val 130 135 140 480 ttt tac tcg gac cgc ttc tgg gta cac ctg gcc agc gcc gtc atg ctg 480 Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu 145 150 155 160 528 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			Met	-		-		Lys			-		Phe		-		384
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		Cys					Ala					Ala					432
Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His 165 170 175	Phe		-	-	-	Phe		-		-	Ala	-	-	-	-	Leu	480
cac cag gtc ttc acc aag cgc aag cac ggg gat ctc gga gga ctc ttt 576					Gln					Leu					Leu		528
	cac	cag	gtc	ttc	acc	aag	cgc	aag	cac	aaa	gat	ctc	gga	gga	ctc	ttt	576

192

His	Gln	Val	Phe 180	Thr	Lys	Arg	Lys	His 185	Gly	Asp	Leu	Gly	Gly 190	Leu	Phe	
	ggg Gly															624
	aac Asn 210															672
	caa Gln															720
	gtc Val			•					•			•	•			768
	tcg Ser															816
	ccc Pro		-	-		-	-	-	-		-					864
-	tgc Cys 290	-					-		-			-	-		-	912
	aag Lys															960
	ctg Leu															1008
	tcg Ser															1056
	gga Gly															1104
	acc Thr 370															1152
	acg Thr															1200
	gac Asp															1248
	ccc Pro															1296
	tcg Ser															1344
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Ala Trp Ile Il 35	e His Ser	Asn Lys 40	Val Ty	vr Asp Val	Ser 45	Asn Trp	His								
Glu His Pro Gl 50	y Gly Ala	Val Ile 55	Phe Th	nr His Ala 60	Gly	Aap Aap	Met								
Thr Asp Ile Ph 65	e Ala Ala 70	Phe His	Ala Pr	o Gly Ser 75	Gln	Ser Leu	Met 80								
Lys Lys Phe Ty	r Ile Gly 85	Glu Leu	Leu Pr 90		Thr	Gly Lys 95	Glu								
Pro Gln Gln Il 10		Glu Lys	Gly Ty 105	r Arg Asp	Leu	Arg Ser 110	ГЛа								
Leu Ile Met Me 115	t Gly Met	Phe Lys 120		en Lys Trp	Phe 125	Tyr Val	Tyr								
Lys Cys Leu Se 130	r Asn Met	Ala Ile 135	Trp Al	a Ala Ala. 140	-	Ala Leu	Val								
Phe Tyr Ser As 145	p Arg Phe 150	Trp Val	His Le	eu Ala Ser 155	Ala	Val Met	Leu 160								
Gly Thr Phe Ph	e Gln Gln 165	Ser Gly	Trp Le 17		Asp	Phe Leu 175	His								
His Gln Val Ph 18	-	Arg Lys	His Gl 185	y Asp Leu	Gly	Gly Leu 190	Phe								
Trp Gly Asn Le 195	ı Met Gln	Gly Tyr 200		ıl Gln Trp	Trp 205	Lys Asn	Lys								
His Asn Gly Hi 210	s His Ala	Val Prc 215	Asn Le	eu His Cys 220		Ser Ala	Val								
Ala Gln Asp Gl 225	y Asp Pro 230	Asp Ile	Asp Th	nr Met Pro 235	Leu	Leu Ala	Trp 240								
Ser Val Gln Gl	n Ala Gln 245	Ser Tyr	Arg Gl 25		Ala	Asp Gly 255	ГЛа								
Asp Ser Gly Le 26	-	Phe Met	Ile Ar 265	g Asn Glr	Ser	Tyr Phe 270	Tyr								
Phe Pro Ile Le 275	ı Leu Leu	Ala Arg 280		er Trp Leu	. Asn 285	Glu Ser	Phe								
Lys Cys Ala Ph 290	e Gly Leu	Gly Ala 295	Ala Se	er Glu Asr 300		Ala Leu	Glu								
Leu Lys Ala Ly 305	s Gly Leu 310		Pro Le	u Leu Glu 315	. Lys	Ala Gly	Ile 320								
Leu Leu His Ty	r Ala Trp 325	Met Leu	Thr Va 33		Gly	Phe Gly 335	Arg								
Phe Ser Phe Al 34	-	Ala Phe	Tyr Ph 345	ie Leu Thr		Thr Ala 350	Ser								
Cys Gly Phe Le 355	ı Leu Ala	Ile Val 360		y Leu Gly	His 365	Asn Gly	Met								
Ala Thr Tyr As 370	n Ala Asp	Ala Arg 375	Pro As	p Phe Trp 380		Leu Gln	Val								
Thr Thr Thr Ar 385	g Asn Val 390	-	Gly Hi	s Gly Phe. 395	Pro	Gln Ala	Phe 400								

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Val	Asp	Trp	Phe	Cys 405	Gly	Gly	Leu	Gln	Tyr 410	Gln	Val	Asp	His	His 415	Leu		
Phe	Pro	Ser	Leu 420	Pro	Arg	His	Asn	Leu 425	Ala	Lys	Thr	His	Ala 430	Leu	Val		
Glu	Ser	Phe 435	Суз	Гла	Glu	Trp	Gly 440	Val	Gln	Tyr	His	Glu 445	Ala	Asp	Leu		
Val	Asp 450	Gly	Thr	Met	Glu	Val 455	Leu	His	His	Leu	Gly 460	Ser	Val	Ala	Gly		
Glu 465	Phe	Val	Val	Asp	Phe 470	Val	Arg	Asp	Gly	Pro 475	Ala	Met					
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atg	gta	ttc	gcg	ggc	ggt											48	
Met 1	Val	Phe	Ala	Gly 5	Gly	Gly	Leu	Gln	Gln 10	Gly	Ser	Leu	Glu	Glu 15	Asn		
					att Ile											96	
					act Thr											144	
					acg Thr											192	
					gct Ala 70											240	
			-		gca Ala	-		-		-		-	-	-		288	
		-		-	aag Lys	-						-	-	-	-	336	
		-		-	gat Asp	-			-	-			-			384	
-	-				gcg Ala	-							-		-	432	
					gac Asp 150											480	
-					att Ile			-					-			528	
			-		cca Pro		_	-		-		-	-	-		576	
-			-		gag Glu					-	-		-			624	

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												gct Ala				720		
												tcc Ser				768		
												gaa Glu	•		000	816		
					-	-	-			-		999 Gly 285			-	864		
	-						-	-			-	tgc Cys	-	-		912		
				-	-	-		-				ctc Leu		-		960		
-	-	-		-	-		-			-		ttc Phe	-	-		1008		
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												ctg Leu				1152		
												ggt Gly				1200		
												atg Met				1248		
												tat Tyr				1296		
												gat Asp 445				1344		
				-								agg Arg				1392		
						-						aac Asn			-	1440		
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Pro Leu Lys Arg 50	Leu Thr Ser 55	Lys Lys A	Arg Val Ser 60	Glu Ser	Ala Ala
Val Gln Cys Ile 65	Ser Ala Glu 70	Val Gln A	Arg Asn Ser 75	Ser Thr	Gln Gly 80
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His Asn Lys Pro 115	Ser Asp Cys	Trp Ile V 120	Val Val Lys	Asn Lys 125	Val Tyr
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Ala Ser Thr Trp	Lys Ile Leu 165		Phe Tyr Ile 170	Gly Asp	Val Glu 175
Arg Val Glu Pro 180		Leu Leu L 185	ys Asp Phe	Arg Glu 190	Met Arg
Ala Leu Phe Leu 195	Arg Glu Gln	Leu Phe L 200	ys Ser Ser	Lys Leu 205	Tyr Tyr
Val Met Lys Leu 210	Leu Thr Asn 215		lle Phe Ala 220	Ala Ser	Ile Ala
Ile Ile Cys Trp 225	Ser Lys Thr 230	Ile Ser A	Ala Val Leu 235	Ala Ser	Ala Cys 240
Met Met Ala Leu	Cys Phe Gln 245		Gly Trp Leu 250	Ser His	Asp Phe 255
Leu His Asn Gln 260		Thr Arg T 265	ſrp Leu Asn	Glu Val 270	Val Gly
Tyr Val Ile Gly 275	Asn Ala Val	Leu Gly P 280	Phe Ser Thr	Gly Trp 285	Trp Lys
Glu Lys His Asn 290	Leu His His 295		Pro Asn Glu 300	Сла Уар	Gln Thr
Tyr Gln Pro Ile 305	Asp Glu Asp 310	Ile Asp T	Thr Leu Pro 315	Leu Ile	Ala Trp 320
Ser Lys Asp Ile	Leu Ala Thr 325		Asn Lys Thr 330	Phe Leu	Arg Ile 335
Leu Gln Tyr Gln 340	His Leu Phe	Phe Met G 345	Gly Leu Leu	Phe Phe 350	Ala Arg
Gly Ser Trp Leu 355	Phe Trp Ser	Trp Arg T 360	fyr Thr Ser	Thr Ala 365	Val Leu
Ser Pro Val Asp	Arg Leu Leu	Glu Lys G	Gly Thr Val	Leu Phe	His Tyr

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Phe	Val	Phe	Val 420	Leu	Ser	His	Asn	Gly 425	Met	Glu	Val	Tyr	Asn 430	Ser	Ser	
Lys	Glu	Phe 435	Val	Ser	Ala	Gln	Ile 440	Val	Ser	Thr	Arg	Asp 445	Ile	Lys	Gly	
Asn	Ile 450	Phe	Asn	Asp	Trp	Phe 455	Thr	Gly	Gly	Leu	Asn 460	Arg	Gln	Ile	Glu	
His 465	His	Leu	Phe	Pro	Thr 470	Met	Pro	Arg	His	Asn 475	Leu	Asn	Lys	Ile	Ala 480	
Pro	Arg	Val	Glu	Val 485	Phe	Сүз	Lys	ГÀа	His 490	Gly	Leu	Val	Tyr	Glu 495	Asp	
Val	Ser	Ile	Ala 500	Thr	Gly	Thr	Суз	Lys 505	Val	Leu	ГЛа	Ala	Leu 510	Lys	Glu	
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Met 1 aaaa Lys gct Ala gct Ala aaaa Lys 65 aga Arg gca Ala ctt	Val tgg Trp gtt Val ttc Phe 50 aag Lys ctt Leu caa	Val ctc Leu att Ile 35 cac His cac His gac Asp gaa Glu gat	Asp tac Tyr 20 gaa Glu gaa Glu gga Gly aaa Lys 100 gat	Lys 5 ctt Leu caa Gln gga gly glu gtt yal aaa Lys gga	Asn agc Ser tat Tyr tct Ser cac His 70 gat Asp atg Met tta	Ala gag Glu aga Arg tct Ser 55 gat Asp atc Ile gtt Val atg	Ser gaa Glu aat Asn 40 cag Gln gaa Glu aat Asn gaa gaa glu	Gly ttgg 25 tcg Ser gct Ala ttc Phe gta Val tca Ser 105 gca	Leu 10 gtg Val gat Asp tat Tyr ctt Leu tca Ser 90 ttc Phe aat	Arg aag Lys gct Ala aag Lys gag Glu 75 gca Ala gaa glu gaa	Met aaa Lys act Thr caa Gln 60 aaa Lys tat Tyr aaaa Lys aca	Lys cat His cat His 45 ctt Leu caa Gln gat Asp cta Leu tat	Val cca Pro 30 att Ile gac Asp ttg Leu gtc Val cga Arg 110 ttc	Asp 15 gga Gly ttc Phe ctt Leu gaa Glu agt ser 95 cag Gln ctg	Gly gga Gly cac His ctg Leu aag gtt Val aag Lys ttt	96 144 192 240 288
Met 1 aaaa Lys gct Ala gct Ala agat Lys 65 aga Arg gca Ala ctt Leu aaa	Val tgg Trp gtt Val ttc Phe 50 aag Lys ctt Leu caa Gln cat	Val ctc Leu att Ile 35 cac His cac His gac Asp gaa Glu gat Asp 115 att	Asp tac Tyr 20 gaa Glu gga Glu gga Gly aaag Lys 100 gat Asp tca	Lys 5 ctt Leu caa Gln gga gGly gag Glu gtt Val s5 aaaa Lys gga gly aca	Asn agc Ser tat Tyr tct Ser cac His 70 gat Asp atg Met ttau ctt	Ala gag Glu aga Arg tct Ser 55 gat Asp atc Ile gtt Val atg Met	Ser gaa Glu aat Asn 40 cag Gln gaa Glu aat Asn gaa Glu aat Lys 120 att	Gly ttg Leu 25 tcg Ser gct Ala ttc Phe gta Val tca Ser 105 gca Ala	Leu 10 gtg Val gat Asp tat Tyr ctt Leu tca Ser 90 ttc Phe aat	Arg aag Lys gct Ala aag Lys Glu 75 gca Ala gaa Glu gaa Glu ttt	Met aaa Lys act Thr caa Gln 60 aaa Lys tat Lys aaa Lys aca Thr gca	Lys cat His cat His 45 ctt Leu caa Gln gat Asp cta Leu tat Tyr 125 ttt	Val cca Pro 30 att Ile gac Asp ttg Leu gtc Val cga Arg 110 ttc Phe tat	Asp 15 gga gly ttc Phe ctt Leu gaa Glu agt Ser 95 cag gln ctg Leu ctt	Gly gga Gly cac His ctg Leu aag Lys 80 gtt Val aag Lys ttt Phe cag	96 144 192 240 288 336

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_	υ	2

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LysAsnArgProLeuAsnArgThrTheSerLeuPhePheGivAsnPhe14tacaaggggttgtcaggggcatggcat<
Leu Gln GIY Phe Ser Arg Asg Trp Trp Lys Asg Lys His Asn Thr His 195 Description of the ser Arg Asg Trp Trp Lys Asg Lys His Asn Thr His 205 Description of the ser Arg Asg Trp Trp Lys Asg Lys His Asn Thr His 205 Description of the ser Arg Asg Trp Trp Lys Asg Cy Asg The Asg Leu Ala 210 Description of the ser Arg Asg Trp Trp Lys Asg Cy Asg The Asg Leu Ala 210 Description of the ser Arg Asg Trp Trp Lys Asg Cy Asg The Asg Leu Ala 210 Description of the ser Arg Asg Trp Cys Tyr Lys Ala Ser 220 Description of the Ala Phe The Pro Gly Asg Leu Cys Lys Tyr Lys Ala Ser 220 Description of the Ala Phe The Pro Gly Asg Leu Cys Lys Tyr Lys Ala Ser 220 Description of the Ala Phe The Pro Gly Asg Leu Cys Lys Tyr Lys Ala Ser 220 Description of the Ala Phe The Pro Gly Asg Leu Cys Lys Tyr Lys Ala Ser 220 Description of the Leu Arg Pro Tyr Gln His Leu Tyr Phe 225 Description of the Leu Arg Phe Ser Trp Thr Gly Gln Ser Val 260 Description of the Leu Arg Phe Ser Trp Thr Gly Gln Ser Val 260 Description of the Leu Arg Phe Ser Trp Thr Gly Gln Ser Val 260 Description of the Lys Vyr Lys Val Tyr Charg 270 Description of the Lys Glu Asg Can Cag at Cag Gt Cag Ca Ca Cag Ca Cag Ca Cag Ca Cag Ca Cas Cas Cas Cag Ca Cag Ca Cas Cas Cas Cas Cas Cas Cas Cas Cas
His Ala Ala Thr Asn Val Ile Asp His Asp Oly Asp Ile Asp Leu Ala 210Yasp Ile Asp Leu Ala 220Yasp Ile Asp Leu Ala 220cca ctt ttc gca ttt att cca gga gat ttg tgc aag tat aag gcc agc 230720cca ctt ttc gca ttt att cca gga gat ttg tgc aag tat aag gcc agc 230720225230230225230240225230240225230240225240780225240780225240780225240780245240780245240780245240780245240780245240780245240780245240780246255780acc ga atg ctt cca atg ctc cgt ttc tca tgg act ggt cag tca gtt 260260260270281270280270280281770283280290280291291291291292291293291294292295290295290296291297291298292298292299292290292291293291293292294293294294295294294295
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Pro Ser Pro Phe Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln Ile 370 375 375 375 380 1200 1200 1200 1200 1200 1200 1200 12
Glu His His Leu Phe Pro Thr Met Pro Arg Cys Asn Leu Asn Ala Cys 385 390 395 400 gtg aaa tat gtg aaa gaa tgg tgc aaa gag aat aat ctt cct tac ctc 1248 Val Lys Tyr Val Lys Glu Trp Cys Lys Glu Asn Asn Leu Pro Tyr Leu
Val Lys Tyr Val Lys Glu Trp Cys Lys Glu Asn Asn Leu Pro Tyr Leu
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<211> LENGTH: 443 <212> TYPE: PRT

<213> ORGANISM: Caenorhabditis elegans

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Pro 225	Leu	Phe	Ala	Phe	Ile 230	Pro	Gly	Asp	Leu	Cys 235	ГЛа	Tyr	Lys	Ala	Ser 240
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210

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Thr Glu

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	Tyr															024
		195					200					205				
cto	acg	cgc	att	cag	ctt	atc	cag	ttc	gtg	acc	atg	aac	gtg	cag	ggc	672
Leu	1 Thr 210	Arg	Ile	Gln	Leu	Ile 215	Gln	Phe	Val	Thr	Met 220	Asn	Val	Gln	Gly	
	210					213					220					
	ctg				-	-	-			-			-		-	720
225	Leu	1111	тут	Ser	230	GIII	сув	PIO	GTY	235	PIO	PIO	цув	vai	240	
	atg Met						-							-		768
		-1-		245	1-				250		r ²			255		
++-		a++	000	aa~	tar	at~	++~	aa		20~	222	~~~	aa	a+ ~	<i></i>	81.6
	tac Tyr		-							-		-	-			816
	_		260		-			265		-	-		270			
qaa	. tcg	aaq	aaa	aaq	tta	taa										837
-	. Ser	Lys	-	-	-											
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His	Pro	Met 35	Ala	Asp	Tyr	Pro	Leu 40	Ala	Asn	Phe	Ser	Ser 45	Val	Tyr	Ala
Ile	Суз 50	Val	Gly	Tyr	Leu	Leu 55	Phe	Val	Ile	Phe	Gly 60	Thr	Ala	Leu	Met
Lys 65	Met	Gly	Val	Pro	Ala 70	Ile	Lys	Thr	Ser	Pro 75	Leu	Gln	Phe	Val	Tyr 80
Asn	Pro	Ile	Gln	Val 85	Ile	Ala	Cys	Ser	Tyr 90	Met	Cys	Val	Glu	Ala 95	Ala
Ile	Gln	Ala	Tyr 100	Arg	Asn	Gly	Tyr	Thr 105	Ala	Ala	Pro	Сүз	Asn 110	Ala	Phe
Lys	Ser	Asp 115	Asp	Pro	Val	Met	Gly 120	Asn	Val	Leu	Tyr	Leu 125	Phe	Tyr	Leu
Ser	Lys 130	Met	Leu	Asp	Leu	Сув 135	Asp	Thr	Val	Phe	Ile 140	Ile	Leu	Gly	Lys
Lys 145	Trp	Lys	Gln	Leu	Ser 150	Ile	Leu	His	Val	Tyr 155	His	His	Leu	Thr	Val 160
Leu	Phe	Val	Tyr	Tyr 165	Val	Thr	Phe	Arg	Ala 170	Ala	Gln	Asp	Gly	Asp 175	Ser
Tyr	Ala	Thr	Ile 180	Val	Leu	Asn	Gly	Phe 185	Val	His	Thr	Ile	Met 190	Tyr	Thr
Tyr	Tyr	Phe 195	Val	Ser	Ala	His	Thr 200	Arg	Asn	Ile	Trp	Trp 205	Lys	Lys	Tyr
Leu	Thr 210	Arg	Ile	Gln	Leu	Ile 215	Gln	Phe	Val	Thr	Met 220	Asn	Val	Gln	Gly
Tyr 225	Leu	Thr	Tyr	Ser	Arg 230	Gln	Суз	Pro	Gly	Met 235	Pro	Pro	Lys	Val	Pro 240
Leu	Met	Tyr	Leu	Val 245	Tyr	Val	Gln	Ser	Leu 250	Phe	Trp	Leu	Phe	Met 255	Asn
Phe	Tyr	Ile	Arg 260	Ala	Tyr	Val	Phe	Gly 265	Pro	Lys	Lys	Pro	Ala 270	Val	Glu
Glu	Ser	Lys 275	Гла	Гла	Leu										
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	gcc Ala														

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222

						acc Thr										96	
						tcc Ser										144	
						gtc Val 55										192	
						cag Gln										240	
	-					ctc Leu					-	-				288	
						ttc Phe										336	
-						gcc Ala		-	-	-		-			-	384	
						tac Tyr 135										432	
						ttg Leu										480	
						tcg Ser										528	
						gtg Val										576	
	-		-		-	tac Tyr				-	-		-	-		624	
						cag Gln 215										672	
						ttc Phe										720	
-						tgg Trp				-		-	-	-		768	
						ttt Phe										816	
-						tac Tyr	-					-	-	-	-	864	
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Thr	Gly	Lys 35	Ser	Ile	Asp	Ser	Phe 40	Val	Phe	Gln	Glu	Gly 45	Val	Thr	Pro
Leu	Ser 50	Thr	Gln	Arg	Glu	Val 55	Ala	Met	Trp	Thr	Ile 60	Thr	Tyr	Phe	Va:
Val 65	Ile	Phe	Gly	Gly	Arg 70	Gln	Ile	Met	Lys	Ser 75	Gln	Asp	Ala	Phe	Ly: 80
Leu	Lys	Pro	Leu	Phe 85	Ile	Leu	His	Asn	Phe 90	Leu	Leu	Thr	Ile	Ala 95	Se:
Gly	Ser	Leu	Leu 100	Leu	Leu	Phe	Ile	Glu 105	Asn	Leu	Val	Pro	Ile 110	Leu	Ala
Arg	Asn	Gly 115	Leu	Phe	Tyr	Ala	Ile 120	Cys	Asp	Asp	Gly	Ala 125	Trp	Thr	Glı
Arg	Leu 130	Glu	Leu	Leu	Tyr	Tyr 135	Leu	Asn	Tyr	Leu	Val 140	ГÀа	Tyr	Trp	Glu
Leu 145	Ala	Asp	Thr	Val	Phe 150	Leu	Val	Leu	Lys	Lys 155	Lys	Pro	Leu	Glu	Ph 16
Leu	His	Tyr	Phe	His 165	His	Ser	Met	Thr	Met 170	Val	Leu	СЛа	Phe	Val 175	Gli
Leu	Gly	Gly	Tyr 180	Thr	Ser	Val	Ser	Trp 185	Val	Pro	Ile	Thr	Leu 190	Asn	Leı
Thr	Val	His 195	Val	Phe	Met	Tyr	Tyr 200	Tyr	Tyr	Met	Arg	Ser 205	Ala	Ala	Gl
Val	Arg 210	Ile	Trp	Trp	Lys	Gln 215	Tyr	Leu	Thr	Thr	Leu 220	Gln	Ile	Val	Glı
Phe 225	Val	Leu	Asp	Leu	Gly 230	Phe	Ile	Tyr	Phe	Cys 235	Ala	Tyr	Thr	Tyr	Phe 240
Ala	Phe	Thr	Tyr	Phe 245	Pro	Trp	Ala	Pro	Asn 250	Val	Gly	ГЛа	Cys	Ala 255	Gl
Thr	Glu	Gly	Ala 260	Ala	Leu	Phe	Gly	Cys 265	Gly	Leu	Leu	Ser	Ser 270	Tyr	Leı
Leu	Leu	Phe 275	Ile	Asn	Phe	Tyr	Arg 280	Ile	Thr	Tyr	Asn	Ala 285	Lys	Ala	Ly:
Ala	Ala 290	Lys	Glu	Arg	Gly	Ser 295	Asn	Phe	Thr	Pro	Lys 300	Thr	Val	Lys	Se:
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48

that atg gac ett goc acc got atc git gtc ggg goc gog coc tat gtc 2096at cot tot gag goc gog ctg gtg gtc goc gag cag atg tat atc coc 150114 25at cot tot gag goc gog ctg gtg gtc cag gcc gag aag tat atc coc 150114 40at get cat cac acg cgt gtg gtg tot ctg gtc gcg gtg gag tog cot 50192at get cat cac acg cgt ggg ttc ctg gtc gcg gtg gag tog cot 50192at get cat cac acg cgt ggg ttc gtg tat at a val Glu Ser Pro 50240at get c got tat gtg co gtg gtg at a gac cg ttc cac gtg ctg ttg atc sta Arg Glu Leu Pro Leu Met Arn Pro Phe His Val Leu Leu I 16240 80at get c got tat ttg gtc acg gtc tt gtg gc atg cag atc atg aag as ttt gag cg gtt gag gtc ag gtc aag acg ttt tog ct ctg tac aac att as s288 90at tt gag cg gt tc gag gtc ag gtc at at gtg gg ggg atg cd gt at at atc cot as s240 90285at tt gag cg gt tc gag gtc ag ct at at gtg cg gt ggg at ctg at at at atg as s288 90288at tt gag cg tt ag at c gg ct tt gtg gg atg gg at ctg at at atg at a set tt gag cgg ttc gag gtc ag acg atg atg atg atg at tt gag cgg ttc gag gtc ag acg atg atg atg atg atg atg atg set less at atg gt ctg atg atg atg atg atg atg atg atg atg a												-	con	tin	ued		
whe Met Asp Leu Ala Thr Ala IL 215 GUY Val Arg Ala Ala Pro Tyr Val 20 at cct ctc gag gcc gc gc gt gt gt gcc asg gcc gag asg tac atc ccc 35 144 up Pro Leu Glu Ala Ala Leu Val Ala Glu Ala Glu Lyg Tyr Ile Pro 35 192 ict ct ctc gag gcc gc gt gt gt gt cc ct g gt gcg gcc gag asg tac atc ccc 36 192 ict gcc ct tc bag gcc gc gt gt gt gt atc ct g gt c gcg gt gg gt gc gcct 192 192 ict gcc cgt gag ctg ccg ttg atg aac ccg ttc cac gtg ctg ttg atc atc acc 36 240 ict gcc cgt tat ttg gc acg gt ct tt gt gcc acg atc atg asg as atc atg asg 36 336 itt gct c gct tat ttg gc acg gt ct atg acg gct tt gg gc atg cag atc atg asg 36 336 itt gag cg gt tc gag gt ca gcg ct ac atg tg gc gt gg gt cc gt gt acg acc 111 336 itt gag cg gt tc gag gcc acg gt ct atg gc gt gg gt gg gc cc ga acc 111 336 itt gag cg gt tc gag gcc acg atc atg tg gg gt cc gt ac acc 111 336 itt gag cg gt tc gat ag acg cct ac atg tg gg gt gc gc tt gt at acc 336 334 itt gag ct gt gt cc atg gcc atg atg atg acc gt gt gt act acc 36 334 itt gag gg gt ct ct at ag gcc ac at gt gag acc gt gt gt act acc 36 432 itt gag gt gt gt gt gt gc ac acc atg tc at acc 37 334 itt gag acc atg gag tt gc gcc atg atg atc tg gt ct ct ac acc 36 432 itt at ag gcg itt gt gc ac ac at gt gt ac at gc acg acc	1				5					10					15		
up Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lyg Tyr Ile Pro 35 192 uog att gtc cat cac acg cgt gg gg ttc dtg gtc gg				Leu					Gly					Pro			96
thr 11e Val His His Thr Arg Glý Phe Leu Val Ala Val Gu ser Pro 50 50 Go Go Ser Pro ttg gec ogt gag otg ogg ttg atg aa cog ttc cac gtg otg ttg atc Gag atc atg aag 240 55 70 70 70 80 240 75 70 70 70 80 240 76 70 70 70 80 288 71 Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys 80 288 76 70 85 90 95 95 70 70 70 70 70 70 70 70 <			Leu					Val					Lys				144
and and any and any and any and any and any and any		Ile					Arg					Ala					192
aiLeu AlaTyrLeu ValThrValPheValGivWetGlnIleMetLyg95acattgagggg ttcgagggg ttgagggg ttggg ttcggg ttggg						Pro					Phe					Ile	240
Ison Phe Glu Arg Phe Glu Val Lyg Thy Phe Ser Leu Leu His Asn Phe 100Ser Leu Leu His Asn Phe 110384gt ctg gtc tcg atc agc gcc tac atg tg cggt ggg atc ctg tac gag 115Ser Ile Ser Ala Tyr Met Cyg Gly Gly Gly Ile Leu Tyr Glu 					Leu					Val					Met		288
Type LeuValSerIleSerÅlaTyrMetCysGlyGlyIleLeuTyrGlu115115110120120125112125112125112111TyrGluAlaAlaAlaAlaAlaAlaAlaAla125111TyrGluLeuPheGluAlaAlaAlaAlaAlaAlaAla130IleProMetAlaLysMetIleTyrPheSer116130IleProMetAlaLysMetIlePheTyrPheSer528145MysIleMetGluPhePheNe				Arg					Thr					His			336
IaTyrGinAlaAsnTyrGlyLeuPheGluAsnAlaAlaAspHisThr130130130140135135135135140AsnAlaAspHisThr140135GlyLeuProAstAlgalg<			Val					Tyr					Ile				384
Phe Lys Giy Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser Aag atc atg gag tt gtc gaa atc atg atc tt full met full met full met full met full met full met full		Tyr					Gly					Ala					432
AysIleMetGluPheValAspThrMetIleMetValLeuLysLysAsnAsccgccagatctccttcttgtggccttgtaccacgacacc <td></td> <td></td> <td></td> <td></td> <td></td> <td>Met</td> <td></td> <td></td> <td></td> <td></td> <td>Trp</td> <td></td> <td></td> <td></td> <td></td> <td>Ser</td> <td>480</td>						Met					Trp					Ser	480
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ThrIleTrpTrpLeuValThrPheValAlaProAsnGlyGluAlaTyr200195195195195195196110 </td <td></td> <td></td> <td></td> <td>Ile</td> <td></td> <td></td> <td></td> <td></td> <td>Val</td> <td></td> <td></td> <td></td> <td></td> <td>Ser</td> <td></td> <td></td> <td>576</td>				Ile					Val					Ser			576
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TyrPheLeuSerAlaLeuGlyPheLysGlnValSerPheLieLysPhe235230230230230230235240240240acaactacgcgcccgcagatgatgatgatgatgatgatgatg240acaactacgcgccagatg <td< td=""><td></td><td>Ser</td><td>•</td><td></td><td></td><td></td><td>Ser</td><td></td><td></td><td></td><td></td><td>Ile</td><td></td><td></td><td></td><td></td><td>672</td></td<>		Ser	•				Ser					Ile					672
TyrIleThrArgSerGlnMetThrGlnPheCysMetMetSerValGlnSertcctgggacatgtacgccatgaaggtccttggccccggatac816SerTrrAspMetTyrAlaMetLysValLeuGlyArgProGlyTyr816SerStrThrAspMetTyrAlaMetLysValLeuGlyArgProGlyTyr864SerThHaLeuLeuLeuTrpPheTyrMetLeu864SerThHaLeuLeuLeuTrpPheTyrMetLeu275Sertcaacattttacagaaagaaggccaagtag912280SerSerMetTyrArgLysAsnAlaLysGln912290SerSerSerSerArgLysAlaArgLysLuAlaLys957200SerSerSerSerSerSerSerSerSerSer957201LysAlaAspAlaAlaLysGluLysAlaArgLysLysLuSer957			-	-	-	Leu			-	-	Val	-			-	Phe	720
Ser Ser TrpAspMetTyrAlaMetLysValLeuGlyArgProGlyTyr260260265265265270270270270864200PheAtcaccaccaccatgtggaccatgctc864275PheFileThrAlaLeuLeuTrpPheTyrMetTrpThrMetLeu275280280280285285285912gtctcttctacaccttt tacagaaagaaggccaagcaggc912gtctcttctacaccttt tacagaaagaaggccaagcag912gtLuPheTyrAspPheTyrArgLysAsnAlaLysGlu957gtaaggccgccgcdgcdaaggcaaggaggtag957gtAlaAspAlaAlaLysGluLysAlaArgLysLeuGlu957					Ser					Phe					Val		768
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu 285 ggt ctc ttc tac aac ttt tac aga aag aac gcc aag ttg gcc aag cag 912 ggt ctc ttc tac aac ttt tac aga aag aag gcc aag ttg gcc aag cag 912 ggt ctc ttc tac aac ttt tac aga aag aag gcc aag ttg gcc aag cag 912 ggt ctc ttc tac aac ttt tac aga aag aag gcc aag ttg gcc aag cag 912 ggt ctc ttc tac aac ttt tac aga aag aag aag aag ttg gcc aag cag 912 ggt cag tag 295 300 gcc aag gcc gcc gct gcc aag gag aag gca agg aag ttg cag taa 957 gla Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln 957				Asp					Lys					Pro			816
Hy Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln 290 295 300 gec aag gec gac get gec aag gag aag gea agg aag ttg cag taa 957 Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln			Phe					Leu					Trp				864
la Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln		Leu					Tyr					Lys					912
						Ala					Arg				taa		957

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Asp H	Pro	Leu 35	Glu	Ala	Ala	Leu	Val 40	Ala	Gln	Ala	Glu	Lys 45	Tyr	Ile	Pro
Thr 1	Ile 50	Val	His	His	Thr	Arg 55	Gly	Phe	Leu	Val	Ala 60	Val	Glu	Ser	Pro
Leu <i>7</i> 65	Ala	Arg	Glu	Leu	Pro 70	Leu	Met	Asn	Pro	Phe 75	His	Val	Leu	Leu	Ile 80
Val I	Leu	Ala	Tyr	Leu 85	Val	Thr	Val	Phe	Val 90	Gly	Met	Gln	Ile	Met 95	Гла
Asn H	Phe	Glu	Arg 100	Phe	Glu	Val	Lys	Thr 105	Phe	Ser	Leu	Leu	His 110	Asn	Phe
Cys I	Leu	Val 115	Ser	Ile	Ser	Ala	Tyr 120	Met	Сув	Gly	Gly	Ile 125	Leu	Tyr	Glu
Ala 1	Fyr 130	Gln	Ala	Asn	Tyr	Gly 135	Leu	Phe	Glu	Asn	Ala 140	Ala	Aab	His	Thr
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Lys]	Ile	Met	Glu	Phe 165	Val	Asp	Thr	Met	Ile 170	Met	Val	Leu	Lys	Lys 175	Asn
Asn A	Arg	Gln	Ile 180	Ser	Phe	Leu	His	Val 185	Tyr	His	His	Ser	Ser 190	Ile	Phe
Thr 1	Ile	Trp 195	Trp	Leu	Val	Thr	Phe 200	Val	Ala	Pro	Asn	Gly 205	Glu	Ala	Tyr
Phe S	Ser 210	Ala	Ala	Leu	Asn	Ser 215	Phe	Ile	His	Val	Ile 220	Met	Tyr	Gly	Tyr
Tyr 1 225	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	Сүз	Met	Met	Ser	Val 255	Gln
Ser S	Ser	Trp	Asp 260	Met	Tyr	Ala	Met	Lys 265	Val	Leu	Gly	Arg	Pro 270	Gly	Tyr
Pro H	Phe	Phe 275	Ile	Thr	Ala	Leu	Leu 280	Trp	Phe	Tyr	Met	Trp 285	Thr	Met	Leu
Gly I 2	Leu 290	Phe	Tyr	Asn	Phe	Tyr 295	Arg	Lys	Asn	Ala	Lүз 300	Leu	Ala	Lys	Gln
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								gtt Val								192	
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				-	-			atc Ile	-		-	-		-		288	
							-	aac Asn 105				-	-			336	
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								tct Ser								528	
								ttt Phe 185								576	
					-	-	-	aag Lys		-						624	
-		-						atc Ile	-						-	672	
								ctc Leu								720	
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Asp Ala Glu Gly 35	Arg Lys Phe	Phe Ala Asp H 40	His Phe Asp Val 45	Thr Ile
Gln Ala Ser Ile 50	Leu Tyr Met 55	Val Val Val P	Phe Gly Thr Lys 60	Trp Phe
Met Arg Asn Arg 65	Gln Pro Phe 70		lle Pro Leu Asn 75	Ile Trp 80
Asn Phe Ile Leu	Ala Ala Phe 85	Ser Ile Ala G 90	Sly Ala Val Lys	Met Thr 95
Pro Glu Phe Phe 100		Ala Asn Lys G 105	Sly Ile Val Ala 110	Ser Tyr
Cys Lys Val Phe 115	Asp Phe Thr	Lys Gly Glu A 120	an Gly Tyr Trp. 125	Val Trp
Leu Phe Met Ala 130	Ser Lys Leu 135		Val Asp Thr Ile 140	Phe Leu
Val Leu Arg Lys 145	Arg Pro Leu 150		His Trp Tyr His .55	His Ile 160
Leu Thr Met Ile	Tyr Ala Trp 165	Tyr Ser His P 170	Pro Leu Thr Pro	Gly Phe 175
Asn Arg Tyr Gly 180		Asn Phe Val V 185	Val His Ala Phe 190	Met Tyr
Ser Tyr Tyr Phe 195	Leu Arg Ser	Met Lys Ile A 200	Arg Val Pro Gly 205	Phe Ile
Ala Gln Ala Ile 210	Thr Ser Leu 215		Sln Phe Ile Ile 220	Ser Cys
Ala Val Leu Ala 225	His Leu Gly 230		His Phe Thr Asn 235	Ala Asn 240
Cys Asp Phe Glu	Pro Ser Val 245	Phe Lys Leu A 250	Ala Val Phe Met	Asp Thr 255
Thr Tyr Leu Ala 260	Leu Phe Val	Asn Phe Phe L 265	Leu Gln Ser Tyr 270	Val Leu
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			ega gag aat gac Arg Glu Asn Asp	

234

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							ccc Pro									480
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							atg Met									912
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													gcg Ala			1200	
													aat Asn			1248	
													gca Ala 430			1296	
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Asn	Gly	Lys	Pro 20	Glu	Asn	Gly	Ala	Thr 25	Pro	Glu	Asn	Gly	Ala 30	Lys	Pro		
Gln	Pro	Суз 35	Glu	Asn	Gly	Thr	Val 40	Glu	Гла	Arg	Glu	Asn 45	Asp	Thr	Ala		
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Asp 65	Ser	Leu	Ala	Val	Ser 70	Gly	Gln	Gly	ГЛа	Glu 75	Arg	Leu	Phe	Thr	Thr 80		
Asp	Glu	Val	Arg	Arg 85	His	Ile	Leu	Pro	Thr 90	Asp	Gly	Trp	Leu	Thr 95	Суз		
His	Glu	Gly	Val 100	Tyr	Asp	Val	Thr	Asp 105	Phe	Leu	Ala	Lys	His 110	Pro	Gly		
Gly	Gly	Val 115	Ile	Thr	Leu	Gly	Leu 120	Gly	Arg	Asp	Суз	Thr 125	Ile	Leu	Ile		

Glu	Ser 130	Tyr	His	Pro	Ala	Gly 135	Arg	Pro	Aab	Lys	Val 140	Met	Glu	Lys	Tyr
Arg 145	Ile	Gly	Thr	Leu	Gln 150	Asp	Pro	Lys	Thr	Phe 155	Tyr	Ala	Trp	Gly	Glu 160
Ser	Asp	Phe	Tyr	Pro 165	Glu	Leu	Lys	Arg	Arg 170	Ala	Leu	Ala	Arg	Leu 175	Lys
Glu	Ala	Gly	Gln 180	Ala	Arg	Arg	Gly	Gly 185	Leu	Gly	Val	ГЛЗ	Ala 190	Leu	Leu
Val	Leu	Thr 195	Leu	Phe	Phe	Val	Ser 200	Trp	Tyr	Met	Trp	Val 205	Ala	His	Lys
Ser	Phe 210	Leu	Trp	Ala	Ala	Val 215	Trp	Gly	Phe	Ala	Gly 220	Ser	His	Val	Gly
Leu 225	Ser	Ile	Gln	His	Asp 230	Gly	Asn	His	Gly	Ala 235	Phe	Ser	Arg	Asn	Thr 240
Leu	Val	Asn	Arg	Leu 245	Ala	Gly	Trp	Gly	Met 250	Asp	Leu	Ile	Gly	Ala 255	Ser
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Asn	Leu	Val 275	Ser	Asp	Thr	Leu	Phe 280	Ser	Leu	Pro	Glu	Asn 285	Asp	Pro	Asp
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Gln 305	Pro	His	His	Arg	Phe 310	Gln	His	Leu	Phe	Ala 315	Phe	Pro	Leu	Phe	Ala 320
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Ser	Met	Lys	Lys 340	Gly	Ser	Ile	Asp	Cys 345	Ser	Ser	Arg	Leu	Val 350	Pro	Leu
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Gln	Ile 370	Val	Leu	Pro	Сүз	Tyr 375	Leu	His	Gly	Thr	Ala 380	Met	Gly	Leu	Ala
Leu 385	Phe	Ser	Val	Ala	His 390	Leu	Val	Ser	Gly	Glu 395	Tyr	Leu	Ala	Ile	Суз 400
Phe	Ile	Ile	Asn	His 405	Ile	Ser	Glu	Ser	Cys 410	Glu	Phe	Met	Asn	Thr 415	Ser
Phe	Gln	Thr	Ala 420	Ala	Arg	Arg	Thr	Glu 425	Met	Leu	Gln	Ala	Ala 430	His	Gln
Ala	Ala	Glu 435	Ala	Lys	Lys	Val	Lys 440	Pro	Thr	Pro	Pro	Pro 445	Asn	Asp	Trp
Ala	Val 450	Thr	Gln	Val	Gln	Cys 455	Cys	Val	Asn	Trp	Arg 460	Ser	Gly	Gly	Val
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Leu	Phe	Pro	Ser	Ile 485	Ser	His	Ala	Asn	Tyr 490	Pro	Thr	Ile	Ala	Pro 495	Val
Val	Lys	Glu	Val 500	Суз	Glu	Glu	Tyr	Gly 505	Leu	Pro	Tyr	Lys	Asn 510	Tyr	Val
Thr	Phe	Trp 515	Asp	Ala	Val	Сув	Gly 520	Met	Val	Gln	His	Leu 525	Arg	Leu	Met
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				gtc ggc aag Val Gly Lys	
				acc ggg ctc Thr Gly Leu 95	
				agg gtg ctc Arg Val Leu 110	
				atg cag cgc Met Gln Arg 125	
				gca ggt ttc Ala Gly Phe	
				ggc ggt gcc Gly Gly Ala	
				gtc gga act Val Gly Thr 175	
				tcg cga ttc Ser Arg Phe 190	
				gcg agt gcg Ala Ser Ala 205	
				tac acc aac Tyr Thr Asn	
				gcc gac gtc Ala Asp Val	-
				ttc agt acg Phe Ser Thr 255	
				ttt tac cac Phe Tyr His 270	

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	ttc Phe		-			-		-		-			-			1104	
	atc Ile 370															1152	
	gct Ala															1200	
	aag Lys	~ ~		~		~	~ ~	-	-	-	-	-	-	-	-	1248	
-	acc Thr	-					-		-	-		-	-	-		1296	
	gtg Val															1344	
	ctc Leu 450															1392	
	gtc Val															1440	
	ggc Gly		-		-	-			-				-			1488	
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45

243

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35

244

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Lys 65	Gly	Val	Pro	Asp	Ala 70	Val	Leu	Arg	Lys	Tyr 75	ГЛЗ	Val	Gly	Lys	Leu 80
Pro	Gln	Gly	Lys	Lys 85	Gly	Glu	Thr	Ser	His 90	Met	Pro	Thr	Gly	Leu 95	Asp
Ser	Ala	Ser	Tyr 100	Tyr	Ser	Trp	Asp	Ser 105	Glu	Phe	Tyr	Arg	Val 110	Leu	Arg
Glu	Arg	Val 115	Ala	Lys	Lys	Leu	Ala 120	Glu	Pro	Gly	Leu	Met 125	Gln	Arg	Ala
Arg	Met 130	Glu	Leu	Trp	Ala	Lys 135	Ala	Ile	Phe	Leu	Leu 140	Ala	Gly	Phe	Trp
Gly 145		Leu	Tyr	Ala	Met 150		Val	Leu	Aap	Pro 155		Gly	Gly	Ala	Met 160
	Ala	Ala	Val	Thr 165		Gly	Val	Phe	Ala 170		Phe	Val	Gly	Thr 175	
Ile	Gln	His	Asp 180		Ser	His	Gly	Ala 185		Ser	Lys	Ser	Arg 190		Met
Asn	Lys	Ala 195		Gly	Trp	Thr	Leu 200		Met	Ile	Gly	Ala 205		Ala	Met
Thr	~		Met	Gln	His		Leu	Gly	His	His			Thr	Asn	Leu
	210 Glu	Met	Glu	Asn		215 Leu	Ala	Гла	Val	-	220 Gly	Ala	Asp	Val	_
225 Pro	Lys	Lys	Val	-	230 Gln	Glu	Ser	Asp		235 Asp	Val	Phe	Ser		240 Tyr
Pro	Met	Leu	-	245 Leu	His	Pro	Trp		250 Arg	Gln	Arg	Phe	-	255 His	Lys
Phe	Gln		260 Leu	Tyr	Ala	Pro	Phe	265 Ile	Phe	Gly	Ser		270 Thr	Ile	Asn
Lys		275 Ile	Ser	Gln	Asp		280 Gly	Val	Val	Leu	Arg	285 Lys	Arg	Leu	Phe
Gln	290 Ile	Asp	Ala	Asn	Суз	295 Arg	Tyr	Gly	Ser	Pro	зоо Trp	Tyr	Val	Ala	Arg
305					310		Thr			315					320
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			340				Leu	345					350		
		355	-	-			360					365			
	370		-			375	Ala		-	-	380		-	-	
Met 385	Ala	Pro	Pro	Arg	Thr 390	Val	His	сту	val	Thr 395	Pro	Met	GIN	val	Thr 400
Gln	Lys	Ala	Leu	Ser 405	Ala	Ala	Glu	Ser	Ala 410	Lys	Ser	Asp	Ala	Asp 415	Lys
Thr	Thr	Met	Ile 420	Pro	Leu	Asn	Asp	Trp 425	Ala	Ala	Val	Gln	Cys 430	Gln	Thr
Ser	Val	Asn 435	Trp	Ala	Val	Gly	Ser 440	Trp	Phe	Trp	Asn	His 445	Phe	Ser	Gly
Gly	Leu 450	Asn	His	Gln	Ile	Glu 455	His	His	Суз	Phe	Pro 460	Gln	Asn	Pro	His

Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala Trp Ser Thr <210> SEQ ID NO 43 <211> LENGTH: 960 <212> TYPE: DNA <213> ORGANISM: Thalassiosira pseudonana <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(960) <223> OTHER INFORMATION: Delta-5 elongase <400> SEOUENCE: 43 atg gtg ttg tac aat gtg gcg caa gtg ctg ctc aat ggg tgg acg gtg Met Val Leu Tyr Asn Val Ala Gln Val Leu Leu Asn Gly Trp Thr Val tat gcg att gtg gat gcg gtg atg a
at aga gac cat ccg ttt att gga Tyr Ala Ile Val Asp Ala Val Met As
n Arg Asp His Pro Phe Ile Gly $\,$ agt aga agt ttg gtt ggg gcg gcg ttg cat agt ggg agc tcg tat gcg Ser Arg Ser Leu Val Gly Ala Ala Leu His Ser Gly Ser Ser Tyr Ala gtg tgg gtt cat tat tgt gat aag tat ttg gag ttc ttt gat acg tat Val Trp Val His Tyr Cys Asp Lys Tyr Leu Glu Phe Phe Asp Thr Tyr ttt atg gtg ttg agg ggg aaa atg gac cag atg gta ctt ggt gaa gtt Phe Met Val Leu Arg Gly Lys Met Asp Gln Met Val Leu Gly Glu Val ggt ggc agt gtg tgg tgt ggc gtt gga tat atg gat atg gag aag atg Gly Gly Ser Val Trp Cys Gly Val Gly Tyr Met Asp Met Glu Lys Met ata cta ctc agc ttt gga gtg cat cgg tct gct cag gga acg ggg aag Ile Leu Leu Ser Phe Gly Val His Arg Ser Ala Gln Gly Thr Gly Lys get tte ace aac aac gtt ace aat eea cat ete acg ett eea eet eat Ala Phe Thr Asn Asn Val Thr Asn Pro His Leu Thr Leu Pro Pro His tct aca aaa aca aaa aaa cag gtc tcc ttc ctc cac atc tac cac cac Ser Thr Lys Thr Lys Lys Gln Val Ser Phe Leu His Ile Tyr His His acg acc ata gcg tgg gca tgg tgg atc gcc ctc cgc ttc tcc ccc ggt Thr Thr Ile Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly gga gac att tac ttc ggg gca ctc ctc aac tcc atc atc cac gtc ctc Gly Asp Ile Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu atg tat tcc tac tac gcc ctt gcc cta ctc aag gtc agt tgt cca tgg Met Tyr Ser Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp aaa cga tac ctg act caa gct caa tta ttg caa ttc aca agt gtg gtg Lys Arg Tyr Leu Thr Gln Ala Gln Leu Leu Gln Phe Thr Ser Val Val gtt tat acg ggg tgt acg ggt tat act cat tac tat cat acg aag cat Val Tyr Thr Gly Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His

CSIRO Exhibit 1013

247	r
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			-			tcg Ser	-	-		-				-	-	816		
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Ser	Arg	Ser 35	Leu	Val	Gly	Ala	Ala 40	Leu	His	Ser	Gly	Ser 45	Ser	Tyr	Ala			
Val	Trp 50	Val	His	Tyr	Суз	Asp 55	Гла	Tyr	Leu	Glu	Phe 60	Phe	Asp	Thr	Tyr			
Phe 65	Met	Val	Leu	Arg	Gly 70	Lys	Met	Asp	Gln	Met 75	Val	Leu	Gly	Glu	Val 80			
Gly	Gly	Ser	Val	Trp 85	Суз	Gly	Val	Gly	Tyr 90	Met	Asp	Met	Glu	Lys 95	Met			
			100		-	Val		105				-	110	-	-			
Ala	Phe	Thr 115	Asn	Asn	Val	Thr	Asn 120	Pro	His	Leu	Thr	Leu 125	Pro	Pro	His			
Ser	Thr 130	Lys	Thr	ГЛЗ	Lys	Gln 135	Val	Ser	Phe	Leu	His 140	Ile	Tyr	His	His			
Thr 145	Thr	Ile	Ala	Trp	Ala 150	Trp	Trp	Ile	Ala	Leu 155	Arg	Phe	Ser	Pro	Gly 160			
Gly	Asp	Ile	Tyr	Phe 165	Gly	Ala	Leu	Leu	Asn 170	Ser	Ile	Ile	His	Val 175	Leu			
Met	Tyr	Ser	Tyr 180	Tyr	Ala	Leu	Ala	Leu 185	Leu	ГЛЗ	Val	Ser	Cys 190	Pro	Trp			
Lys	Arg	Tyr 195	Leu	Thr	Gln	Ala	Gln 200	Leu	Leu	Gln	Phe	Thr 205	Ser	Val	Val			
Val	Tyr 210	Thr	Gly	Суз	Thr	Gly 215	Tyr	Thr	His	Tyr	Tyr 220	His	Thr	Lys	His			
Gly 225	Ala	Asp	Glu	Thr	Gln 230	Pro	Ser	Leu	Gly	Thr 235	Tyr	Tyr	Phe	Суз	Cys 240			

250

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Gly	Val	Gln	Val	Phe 245	Glu	Met	Val	Ser	Leu 250	Phe	Val	Leu	Phe	Ser 255	Ile	
Phe	Tyr	Lys	Arg 260	Ser	Tyr	Ser	Lys	Lys 265	Asn	Lys	Ser	Gly	Gly 270	Lys	Asp	
Ser	ГЛЗ	Lys 275	Asn	Asp	Asp	Gly	Asn 280	Asn	Glu	Asp	Gln	Cys 285	His	Lys	Ala	
Met	Lys 290	Asp	Ile	Ser	Glu	Gly 295	Ala	Lys	Glu	Val	Val 300	Gly	His	Ala	Ala	
Lуз 305	Asp	Ala	Gly	Lys	Leu 310	Val	Ala	Thr	Arg	Val 315	Arg	СЛа	Lys	Val		
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													tgg Trp			480
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Trp	Leu	Суз 35	Asp	Phe	Arg	Ser	Ala 40	Ile	Thr	Ile	Ala	Leu 45	Ile	Tyr	Ile			
Ala	Phe 50	Val	Ile	Leu	Gly	Ser 55	Ala	Val	Met	Gln	Ser 60	Leu	Pro	Ala	Met			
Asp 65	Pro	Tyr	Pro	Ile	Lys 70	Phe	Leu	Tyr	Asn	Val 75	Ser	Gln	Ile	Phe	Leu 80			
Сүз	Ala	Tyr	Met	Thr 85	Val	Glu	Ala	Gly	Phe 90	Leu	Ala	Tyr	Arg	Asn 95	Gly			
Tyr	Thr	Val	Met 100	Pro	Сүз	Asn	His	Phe 105	Asn	Val	Asn	Asp	Pro 110	Pro	Val			
Ala	Asn	Leu 115	Leu	Trp	Leu	Phe	Tyr 120	Ile	Ser	Lys	Val	Trp 125	Asp	Phe	Trp			
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Leu 145	His		Tyr		His 150			Ile				Tyr	-		Asn 160			
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Thr	Lys	Asp 195	Ser	Lys	Thr	Gly	Lys 200	Ser	Leu	Pro	Ile	Trp 205	Trp	Lys	Ser			
Ser	Leu 210	Thr	Ala	Phe	Gln	Leu 215	Leu	Gln	Phe	Thr	Ile 220	Met	Met	Ser	Gln			
Ala 225	Thr	Tyr	Leu	Val	Phe 230	His	Gly	Суз	Asp	Lys 235	Val	Ser	Leu	Arg	Ile 240			
Thr	Ile	Val	Tyr	Phe 245	Val	Ser	Leu	Leu	Ser 250	Leu	Phe	Phe	Leu	Phe 255	Ala			
Gln	Phe	Phe	Val 260	Gln	Ser	Tyr	Met	Ala 265	Pro	Lys	Lys	Lys	Lys 270	Ser	Ala			

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	gtc Val															144
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	Leu															
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cag	cac	ggc	tac	atg	gtt	gcg	gtg	gac	cgt	tgc	ttc	gct	gct	tgg	aac	240
	His	Gly	Tyr	Met		Ala	Val	Asp	Arg		Phe	Ala	Ala	Trp		
65					70					75					80	
	gct															288
Leu	Ala	Leu	Ser	Val 85	Phe	Ser	Thr	Trp	Gly 90	Phe	Tyr	His	Met	Ala 95	Val	
				05					50					55		
	ctc															336
GIY	Leu	IÀT	100	Met	TUL	GIU	TUT.	105	GTÀ	Leu	GTU	Pne	110	тте	сув	
	tcg Ser						-			_					-	384
011	201	115	011	014	Doa		120		204	0111		125	110			
ata	~~~	ata	+ ~ ~	ata	++ a	+	++ a			ata		<i></i>	++~	ata	<i>a</i>	432
	gcg Ala															432
	130					135					140					
acq	gtg	ttt	ctc	atc	ctq	aaq	qcc	aaq	aaq	qtc	cqc	ttc	ttq	caq	tqq	480
Thr	Val				Leu					Val					Trp	
145					150					155					160	
	cac															528
Tyr	His	His	Ala	Thr 165	Val	Met	Leu	Phe	Cys 170	Trp	Leu	Ala	Leu	Ala 175	Thr	
				100					1,0					1/5		
	tac															576
GIU	Tyr	Inr	180	GIY	Leu	Trp	Pne	A14 185	AIA	Inr	Asn	Tyr	Pne 190	vai	HIS	
	atc Ile															624
		195	-1-		-1-		200					205				
aad	gtg	ata	aad	acc	ato	acc	cct	ata	ato	aca	att	ato	cad	att	act	672
	Val															0,12
	210					215					220					
caq	atg	qtc	tqq	qqc	ctc	atc	qtc	aac	qqc	atc	qcc	atc	acc	acc	ttc	720
	Met															
225					230					235					240	
ttc	acg	act	qqt	qcc	tqc	caq	atc	caq	tct	qtq	act	qtq	tat	tcq	qcc	768
	Thr															
				245					250					255		
atc	atc	ata	tac	qct	tca	tac	ttc	tac	cta	ttc	tcc	caq	ctc	ttc	ttc	816
	Ile	-	Tyr	-	-			Tyr	-			-	Leu			
			260					265					270			
gaq	gcc	cat	ggt	gcc	gct	ggc	aaq	aac	aaq	aaq	aaq	tta	acc	cạc	gaq	864
55	5-			<u> </u>	<u> </u>		5	. =				J	-	5-	5 5	

Glu	Ala	His 275	Gly	Ala	Ala	Gly	Lys 280	Asn	Lys	Lys	Lys	Leu 285	Thr	Arg	Glu	
					tcg Ser											912
	Lys				gtg Val 310		tga									936
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Met 1	Ser	Ala	Phe	Met 5	Thr	Leu	Pro	Gln	Ala 10	Leu	Ser	Asp	Val	Thr 15	Ser	
Ala	Leu	Val	Thr 20	Leu	Gly	Lys	Asp	Val 25	Ser	Ser	Pro	Ser	Ala 30	Phe	Gln	
Ala	Val	Thr 35	Gly	Phe	СЛа	Arg	Glu 40	Gln	Trp	Gly	Ile	Pro 45	Thr	Val	Phe	
Сүз	Leu 50	Gly	Tyr	Leu	Ala	Met 55	Val	Tyr	Ala	Ala	Arg 60	Arg	Pro	Leu	Pro	
Gln 65	His	Gly	Tyr	Met	Val 70	Ala	Val	Asp	Arg	Сув 75	Phe	Ala	Ala	Trp	Asn 80	
Leu	Ala	Leu	Ser	Val 85	Phe	Ser	Thr	Trp	Gly 90	Phe	Tyr	His	Met	Ala 95	Val	
Gly	Leu	Tyr	Asn 100	Met	Thr	Glu	Thr	Arg 105	Gly	Leu	Gln	Phe	Thr 110	Ile	Суз	
Gly	Ser	Thr 115		Glu	Leu	Val	Gln 120	Asn	Leu	Gln	Thr	Gly 125	Pro	Thr	Ala	
Leu	Ala 130	Leu	Суз	Leu	Phe	Cys 135	Phe	Ser	Lys	Ile	Pro 140	Glu	Leu	Met	Asp	
Thr 145	Val	Phe	Leu	Ile	Leu 150	Lys	Ala	Lys	Lys	Val 155	Arg	Phe	Leu	Gln	Trp 160	
Tyr	His	His	Ala	Thr 165	Val	Met	Leu	Phe	Cys 170	Trp	Leu	Ala	Leu	Ala 175	Thr	
Glu	Tyr	Thr	Pro 180	Gly	Leu	Trp	Phe	Ala 185	Ala	Thr	Asn	Tyr	Phe 190	Val	His	
Ser	Ile	Met 195	Tyr	Met	Tyr	Phe	Phe 200	Leu	Met	Thr	Phe	Lys 205	Ser	Ala	Ala	
ГЛа	Val 210	Val	Lys	Pro	Ile	Ala 215	Pro	Leu	Ile	Thr	Val 220	Ile	Gln	Ile	Ala	
Gln 225	Met	Val	Trp	Gly	Leu 230	Ile	Val	Asn	Gly	Ile 235	Ala	Ile	Thr	Thr	Phe 240	
Phe	Thr	Thr	Gly	Ala 245	СЛа	Gln	Ile	Gln	Ser 250	Val	Thr	Val	Tyr	Ser 255	Ala	
Ile	Ile	Met	Tyr 260	Ala	Ser	Tyr	Phe	Tyr 265	Leu	Phe	Ser	Gln	Leu 270	Phe	Phe	
Glu	Ala	His 275	Gly	Ala	Ala	Gly	Lys 280	Asn	Lys	Lys	Lys	Leu 285	Thr	Arg	Glu	
Leu	Ser 290	Arg	Lys	Ile	Ser	Glu 295	Ala	Leu	Leu	Asn	Thr 300	Gly	Asp	Glu	Val	
Ser 305	Lys	His	Leu	Lys	Val 310	Asn										

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<pre><210> SEQ ID NC <211> LENGTH: 9 <212> TYPE: DNA <213> ORGANISM: <220> FEATURE: <221> NAME/KEY: <222> LOCATION: <223> OTHER INF</pre>	Crypthecodin CDS (1)(927)	5e		
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act ttg aac cac Thr Leu Asn His 20			al Val Thr	96
cag ttc tgc agg Gln Phe Cys Arg 35				144
tac ttg gca atg Tyr Leu Ala Met 50				192
tac atg tct ctc Tyr Met Ser Leu 65				240
tcg ctc ttc agt Ser Leu Phe Ser				288
cac acc act tgg His Thr Thr Trp 100	Asn Phe Gly	Thr Ile Cys G		336
acg gag ctt gtg Thr Glu Leu Val 115				384
atc ctg ttc tgc Ile Leu Phe Cys 130				432
ttg atc ttg aag Leu Ile Leu Lys 145				480
acg acc gtg atg Thr Thr Val Met				528
cct gga ttg tgg Pro Gly Leu Trp 180	Phe Ala Ala	Phe Val His S		576
tac atg tac tto Tyr Met Tyr Phe 195				624
aag ccc atc gcg Lys Pro Ile Ala 210		-		672
tgg ggc ttg gtc Trp Gly Leu Val 225		 		720
ggc aac tgc cag Gly Asn Cys Gln				768
tac gcc tcc tac Tyr Ala Ser Tyr 260	Phe Tyr Leu	 Leu Phe Phe G		816

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											-	con	tin	ued		
							aag Lys 280									864
							gca Ala									912
		gtg Val		tga												927
<211 <212	l> LI 2> T?	EQ II ENGTH YPE : RGANI	H: 3 PRT	08	othe	codiı	nium	cohi	nii							
		EQUEI			-											
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Thr	Leu	Asn	His 20	Asp	Phe	Ser	Ser	Val 25	Glu	Pro	Phe	Lys	Val 30	Val	Thr	
Gln	Phe	Сув 35	Arg	Asp	Gln	Trp	Ala 40	Ile	Pro	Thr	Val	Phe 45	Cys	Ile	Gly	
-	50				-	55	Thr	-			60		-			
65					70	-	Cys			75	-			-	80	
				85	-	-	Phe	-	90				-	95		
			100			-	Leu Gln	105				-	110			
		115			-		120 Ile	-	-			125				
	130 Ile	Leu	Lys	Gly		135 Lys	Val	Arg	Phe		140 Gln	Trp	Tyr	His		
145 Thr	Thr	Val	Met		150 Phe	Суз	Trp	Met		155 Leu	Ala	Thr	Glu	-	160 Thr	
Pro	Gly	Leu	Trp 180	165 Phe	Ala	Ala	Thr	Asn 185	170 Tyr	Phe	Val	His	Ser 190	175 Ile	Met	
Tyr	Met	Tyr 195		Phe	Leu	Met	Thr 200		Гуз	Thr	Ala	Ala 205		Ile	Ile	
Lys	Pro 210	Ile	Ala	Pro	Leu	Ile 215	Thr	Ile	Ile	Gln	Ile 220	Ser	Gln	Met	Val	
Trp 225	Gly	Leu	Val	Val	Asn 230	Ala	Ile	Ala	Val	Gly 235	Thr	Phe	Phe	Thr	Thr 240	
Gly	Asn	Суз	Gln	Ile 245	Gln	Ala	Val	Thr	Val 250	Tyr	Ser	Ala	Ile	Val 255	Met	
Tyr	Ala	Ser	Tyr 260	Phe	Tyr	Leu	Phe	Gly 265	Gln	Leu	Phe	Phe	Glu 270	Ala	Gln	
Gly	Ser	Ala 275	Gly	Lys	Asp	Lys	Lys 280	-	Leu	Ala	Arg	Glu 285	Leu	Ser	Arg	
Lys	Val 290	Ser	Arg	Ala	Leu	Thr 295	Ala	Thr	Gly	Glu	Glu 300	Val	Ser	Lys	His	
Met	Lys	Val	Asn													

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<210> SEQ ID NO 51 <211> LENGTH: 795 <212> TYPE: DNA <213> ORGANISM: Oncorhynchus mykiss <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(795) <223> OTHER INFORMATION: Delta-5 elongase <400> SEQUENCE: 51 atg gct tca aca tgg caa agc gtt cag tcc atg cgc cag tgg att tta Met Ala Ser Thr Trp Gln Ser Val Gln Ser Met Arg Gln Trp Ile Leu gag aat gga gat aaa agg aca gac cca tgg cta ctg gtc tac tcc cct Glu Asn Gly Asp Lys Arg Thr Asp Pro Trp Leu Leu Val Tyr Ser Pro atg cca gtg gcc att ata ttc ctc ctc tat ctt ggt gtg gtc tgg gct Met Pro Val Ala Ile Ile Phe Leu Leu Tyr Leu Gly Val Val Trp Ala ggg ccc aag ctg atg aaa cgc agg gaa cca gtt gat ctc aag gct gta Gly Pro Lys Leu Met Lys Arg Arg Glu Pro Val Asp Leu Lys Ala Val ctc att gtc tac aac ttc gcc atg gtc tgc ctg tct gtc tac atg ttc Leu Ile Val Tyr Asn Phe Ala Met Val Cys Leu Ser Val Tyr Met Phe cat gag ttc ttg gtc acg tcc ttg ctg tct aac tac agt tac ctg tgt His Glu Phe Leu Val Thr Ser Leu Leu Ser Asn Tyr Ser Tyr Leu Cys caa cct gtg gat tac agc act agt cca ctg gcg atg agg atg gcc aaa Gln Pro Val Asp Tyr Ser Thr Ser Pro Leu Ala Met Arg Met Ala Lys gta tgc tgg tgg ttt ttc ttc tcc aag gtc ata gaa ttg gct gac acg Val Cys Trp Trp Phe Phe Phe Ser Lys Val Ile Glu Leu Ala Asp Thr gtg ttc ttc atc ctg agg aag aag aac agt cag ctg act ttc ctg cat Val Phe Phe Ile Leu Arg Lys Lys Asn Ser Gln Leu Thr Phe Leu His gtc tat cac cat ggc acc atg atc ttc aac tgg tgg gca ggg gtc aag Val Tyr His His Gly Thr Met Ile Phe Asn Trp Trp Ala Gly Val Lys tat ctg gct gga ggc caa tcg ttc ttc atc ggc ctg ctc aat acc ttt Tyr Leu Ala Gly Gly Gln Ser Phe Phe Ile Gly Leu Leu Asn Thr Phe gtg cac atc gtg atg tac tct tac tac gga ctg gct gcc ctg ggg cct Val His Ile Val Met Tyr Ser Tyr Tyr Gly Leu Ala Ala Leu Gly Pro cac acg cag aag tac tta tgg tgg aag cgc tat ctg acc tca ctg cag His Thr Gln Lys Tyr Leu Trp Trp Lys Arg Tyr Leu Thr Ser Leu Gln ctg ctc cag ttt gtc ctg ttg acc act cac act ggc tac aac ctc ttc Leu Leu Gln Phe Val Leu Leu Thr Thr His Thr Gly Tyr Asn Leu Phe act gag tgt gac ttc ccg gac tcc atg aac gct gtg gtg ttt gcc tac Thr Glu Cys Asp Phe Pro Asp Ser Met Asn Ala Val Val Phe Ala Tyr tgt gtc agt ctc att gct ctc ttc agc aac ttc tac tat cag agc tac Cys Val Ser Leu Ile Ala Leu Phe Ser Asn Phe Tyr Tyr Gln Ser Tyr ctc aac agg aag agc aag aag aca taa

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Leu Asn Arg Lys Ser Lys Lys Thr 260

<210> SEQ ID NO 52 <211> LENGTH: 264 <212> TYPE: PRT <213> ORGANISM: Oncorhynchus mykiss															
<400> SEQUENCE:	1														
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Glu Asn Gly Asp 20	Lys Arg Thr	Asp Pro I 25	Irp Leu Leu	Val Tyr S 30	er Pro										
Met Pro Val Ala 35	Ile Ile Phe	Leu Leu I 40		Val Val T 45	rp Ala										
Gly Pro Lys Leu 50	Met Lys Arg 55	Arg Glu P	Pro Val Asp 60	Leu Lys A	la Val										
Leu Ile Val Tyr 65	Asn Phe Ala 70	Met Val C	Cys Leu Ser 75	Val Tyr M	et Phe 80										
His Glu Phe Leu	Val Thr Ser 85		Ser Asn Tyr 90	-	eu Cys 5										
Gln Pro Val Asp 100	Tyr Ser Thr	Ser Pro L 105	Leu Ala Met .	Arg Met A 110	la Lys										
Val Cys Trp Trp 115	Phe Phe Phe	Ser Lys V 120		Leu Ala A 125	sp Thr										
Val Phe Phe Ile 130	Leu Arg Lys 135		Ser Gln Leu 140	Thr Phe L	eu His										
Val Tyr His His 145	Gly Thr Met 150	Ile Phe A	Asn Trp Trp . 155	Ala Gly V	al Lys 160										
Tyr Leu Ala Gly	Gly Gln Ser 165		Ile Gly Leu 170		hr Phe 75										
Val His Ile Val 180	Met Tyr Ser	Tyr Tyr G 185	Gly Leu Ala .	Ala Leu G 190	ly Pro										
His Thr Gln Lys 195	Tyr Leu Trp	Trp Lys A 200		Thr Ser L 205	eu Gln										
Leu Leu Gln Phe 210	Val Leu Leu 215	Thr Thr H	His Thr Gly 220	Tyr Asn L	eu Phe										
Thr Glu Cys Asp 225	Phe Pro Asp 230	Ser Met A	Asn Ala Val 235	Val Phe A	la Tyr 240										
Cys Val Ser Leu	Ile Ala Leu 245		Asn Phe Tyr 250	-	er Tyr 55										
Leu Asn Arg Lys 260	Ser Lys Lys	Thr													
<pre><211> LENGTH: 88 <212> TYPE: DNA <213> ORGANISM: <220> FEATURE: <220> FEATURE: <221> NAME/KEY: <222> LOCATION: <223> OTHER INFC <400> SEQUENCE:</pre>	<210> SEQ ID NO 53 <211> LENGTH: 885 <212> TYPE: DNA <213> ORGANISM: Oncorhynchus mykiss														
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ggt ccc aga gat	gag cgg gta	cag gga t	tgg ctg ctt	ctg gac a	ac tac										

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C1v																	
ULY	Pro	Arg	Asp 20	Glu	Arg	Val	Gln	Gly 25	Trp	Leu	Leu	Leu	Asp 30	Asn	Tyr		
					cta Leu											144	
		-		-	aga Arg		-	-	-			-				192	
	-	-			ctg Leu 70			-		-				-		240	
					gct Ala											288	
	-			-	gca Ala		-		-		-					336	
-					ttc Phe		-					-	-			384	
					aag Lys											432	
					atg Met 150											480	
					tcc Ser											528	
					tct Ser											576	
					tgg Trp	-						-	-	-		624	
					atg Met						•	•				672	
					999 Gly 230											720	
					ttc Phe											768	
					aag Lys											816	
					gtg Val											864	
	299	aaa			ggg	gac Asp										885	

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												0011	0 1 11		
Met 1	Glu	Thr	Phe	Asn 5	Tyr	Lys	Leu	Asn	Met 10	Tyr	Ile	Asp	Ser	Trp 15	Met
Gly	Pro	Arg	Asp 20	Glu	Arg	Val	Gln	Gly 25	Trp	Leu	Leu	Leu	Asp 30	Asn	Tyr
Pro	Pro	Thr 35	Phe	Ala	Leu	Thr	Val 40	Met	Tyr	Leu	Leu	Ile 45	Val	Trp	Met
Gly	Pro 50	Lys	Tyr	Met	Arg	His 55	Arg	Gln	Pro	Val	Ser 60	Суз	Arg	Gly	Leu
Leu 65	Leu	Val	Tyr	Asn	Leu 70	Gly	Leu	Thr	Ile	Leu 75	Ser	Phe	Tyr	Met	Phe 80
Tyr	Glu	Met	Val	Ser 85	Ala	Val	Trp	His	Gly 90	Asp	Tyr	Asn	Phe	Phe 95	Cys
Gln	Asp	Thr	His 100	Ser	Ala	Gly	Glu	Thr 105	Asp	Thr	Lys	Ile	Ile 110	Asn	Val
Leu	Trp	Trp 115	Tyr	Tyr	Phe	Ser	Lys 120	Leu	Ile	Glu	Phe	Met 125	Aab	Thr	Phe
Phe	Phe 130	Ile	Leu	Arg	Lys	Asn 135	Asn	His	Gln	Ile	Thr 140	Phe	Leu	His	Ile
Tyr 145	His	His	Ala	Ser	Met 150	Leu	Asn	Ile	Trp	Trp 155	Phe	Val	Met	Asn	Trp 160
Val	Pro	Суз	Gly	His 165	Ser	Tyr	Phe	Gly	Ala 170	Ser	Leu	Asn	Ser	Phe 175	Ile
His	Val	Leu	Met 180	Tyr	Ser	Tyr	Tyr	Gly 185	Leu	Ser	Ala	Val	Pro 190	Ala	Leu
Arg	Pro	Tyr 195	Leu	Trp	Trp	Lys	Lys 200	Tyr	Ile	Thr	Gln	Val 205	Gln	Leu	Ile
Gln	Phe 210	Phe	Leu	Thr	Met	Ser 215	Gln	Thr	Ile	Сув	Ala 220	Val	Ile	Trp	Pro
Сув 225	Asp	Phe	Pro	Arg	Gly 230	Trp	Leu	Tyr	Phe	Gln 235	Ile	Phe	Tyr	Val	Ile 240
Thr	Leu	Ile	Ala	Leu 245	Phe	Ser	Asn	Phe	Tyr 250	Ile	Gln	Thr	Tyr	Lys 255	Lys
His	Leu	Val	Ser 260	Gln	Lys	ГЛа	Glu	Tyr 265	His	Gln	Asn	Gly	Ser 270	Val	Ala
Ser	Leu	Asn 275	Gly	His	Val	Asn	Gly 280	Val	Thr	Pro	Thr	Glu 285	Thr	Ile	Thr
His	Arg 290	Lys	Val	Arg	Gly	Asp 295									
<21: <21: <22: <22: <22: <22: <22: <400 acgg	L> LI 2> T 3> OF 0> FI L> NZ 2> L(3> O 3> O SI 0> SI	ENGTI (PE : RGAN: SATUI AME / I DCAT: DCAT: THER SQUEI	ISM: RE: KEY: ION: INF(NCE: agccs	753 Onco CDS (51: ORMA 55 gccga	3) FION	(139 : De: gggt	7) lta-! gacaç	5 elo g cco	etec	gaag					gtgcgt 60 ctccga 120
acaa	ataaa	aga 1	ttcta	acaat	ca ci	cage	ttta	a tg	gttat	gaa	gago	gaaaa	aat t	ggca	agtaac 180
															atgega 240 atetat 300
ccay	,					-233;	Juan	u		, e gu	~30;	y		90	

269

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												COII		ucu		
taaca	agat	at	ataa	atgca	aa a	aact	gcatt	aad	ccact	tta	act	aata	ctt	tcaad	catttt	360
cggtt	tgt	at	tact	tctta	at to	caaat	tgtaa	a taa	aaagt	tatc	aac	aaaaa	aat	tgtta	aatata	420
cctct	tata	ct	ttaa	cgtca	aa g	gaga	aaaaa	a cco	ccgga	atcg	gac	tacta	agc	agcto	gtaata	480
cgact	cac	ta	tagg	gaata	at ta	aagci	ttaca	a ta						tat Tyr		533
cta a Leu A		-			-			-			-	-			-	581
cag g Gln C 2																629
gtc a Val M 40																677
aga d Arg (-	-			-					_	-			-		725
ctc a Leu 1																773
tgg d Trp H																821
gaa a Glu 1 1																869
aag d Lys I 120																917
aac c Asn H																965
aac a Asn 1																1013
ttt g Phe G																1061
tat g Tyr (1																1109
aaa t Lys J 200					-	-	-		-			-		-		1157
cag a Gln 1	-		-	-	-				-	-			-			1205
ctg t Leu 1			-				-					-				1253
aac t Asn F																1301
gag t Glu 1 2			-				-	-		-						1349
aaa a	gtg	aca	ccc	acg	gaa	acc	att	aca	cac	agg	aaa	gtg	agg	<u>aaa</u>	gac	1397

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280 285 290 295 tgaaggatcc actagtaacg gccgccagtg tgctggaatt ctgcagatat ccagcaca ggcggccgct cgagtctaga gggcccttcg aaggtaagcc tatccctaac cctctcct	cg 1517 at 1577
ggeggeeget egagtetaga gggeeetteg aaggtaagee tateeetaae eeteteet	cg 1517 at 1577
	at 1577
gtctcgattc tacgcgtacc ggtcatcatc accatcacca ttgagtttaa acccgctg	ac 1637
cctagagggc cgcatcatgt aattagttat gtcacgctta cattcacgcc ctcccccc	ao 100,
atccgctcta accgaaaagg aaggagttag acaacctgaa gtctaggtcc ctatttat	tt 1697
ttttatagtt atgttagtat taagaacgtt atttatattt caaatttttc ttttttt	ct 1757
gtacagacgc gtgtacgcat gtaacattat actgaaaacc ttgcttgaga aggttttg	gg 1817
acgetegaag getttaattt geaagetgeg geeetgeatt aatgaategg eeaaegeg	cg 1877
gggagaggcg gtttgcgtat tgggcgctct tccgcttcct cgctcactga ctcgctgc	gc 1937
teggtegtte ggetgeggeg ageggtatea geteacteaa aggeggtaat aeggttat	cc 1997
acagaatcag gggataacgc aggaaagaac atgtgagcaa aaggccagca aaagccca	gg 2057
aaccgtaaaa aggccgcgtt gctggcgttt ttccataggc tccgcccccc tgacgagc	at 2117
cacaaaaatc gacgctcaag tcagaggtgg cgaaacccga caggactata aagatacc	ag 2177
gegttteeee etggaagete eetegtgege teteetgtte egaceetgee gettaeeg	ga 2237
tacetgteeg eettteteee ttegggaage gtggegettt eteatagete aegetgta	gg 2297
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cagecegaee getgegeett atceggtaae tategtettg agtecaaeee ggtaagae	ac 2417
gacttatcgc cactggcagc agccactggt aacaggatta gcagagcgag gtatgtag	gc 2477
ggtgctacag agttettgaa gtggtggeet aaetaegget aeaetagaag gaeagtat	tt 2537
ggtatetgeg etetgetgaa gecagttaee tteggaaaaa gagttggtag etettgat	
ggcaaacaaa ccaccgctgg tagcggtggt ttttttgttt gcaagcagca gattacgc	
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aacgaaaact cacgttaagg gattttggtc atgagattat caaaaaggat cttcacct	
atcettttaa attaaaaatg aagttttaaa teaatetaaa gtatatatga gtaaaett	
tetgacagtt accaatgett aatcagtgag geacetatet eagegatetg tetattte	
tcatccatag ttgcctgact ccccgtcgtg tagataacta cgatacggga gcgcttac	
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sase to t ttg geg to t tt geg ang to t tt geg dig to geg geg 480 as at gg gab c ang get to t the dec and ta ge dat the at gg dig to geg geg and the set of the dec and geg and the set of the dec and geg and the set of the set of the dec and geg and the set of th													0011	CIII	ucu		
Lyo Net Âep Qin vial Ser Phe Leu His 11e Tyr His His Thr Thr 11e 165 165 165 167 168 169 169 169 160 160 160 160 160 160 160 160	Asp	Lys				Phe					Phe					Gly	480
Alà Trỹ trỹ trỹ tle Ala Leu Arg Phe Ser Pro Cly Cly Agg 11e 130 130 130 130 130 130 130 130 130 130 130 140 141 142 143 144 145 145 146 147 148 148 149 141 144 144 145 145 146 146 147 148 148 149 141 141 141 142 141 141 142 143 144 145 145 146 147 148 149 141 141					Val					Ile					Thr		528
Tyr Phe GTY Ala Leu Leu Ann Ser Ile 11e His Val Leu Mei Tyr Ser 135 136 137 Tyr Tyr Ala Leu Lau Leu Leu Lye Val Ser Cyp Pro Trp Lye Arg Tyr 210 Ctg act cas get cas that tig cas the aca agt gig gig gig git tat acg Leu Thr Gin Ala Gin Leu Leu Gin Phe Thr Ser Val Val Val Tyr Thr 228 229 229 220 230 240 240 240 240 240 240 241 241 241 241 242 240 240 240 245 245 245 246 247 246 247 247 247 247 248 249 249 249 249 249 249 249 249			-	Trp			-		Arg					Gly	-		576
Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp Lys Arg Tyr 210720Ctg act caa got caa tha the g caa the aca agt gitg gtg gth tat acg 225720ggg tgt acg ggt tat act cat tac tac tac cat acg aag cat gga gcg gat G1V Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His Gly Ala App 245768ggg tgt acg ggt tat act cat tac tat cat tac ta to ta to tag aag cat gga gtg cag G10 Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Dry Phe Cys Cys Gly Val Gln 260816ggt tt gag atg gtt agt tt gga acg tat tat the tot gt tg gga gtg cag G10 Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln 260816ggt gtt taga atg gtt agt tt gt tt gt act cht the cat the tat aaa Val Phe CTO 275864gat cat tat cg aag aag aac aag tca gga gga aag gat agc aag aag Arg Ser Tyr Ser Lys Lys Apn Lys Ser Gly Gly Lys App Ser Lys Lys 200912aat gat gat ggg aat aat gag gat caa tgt cac ag gt ca ga gg at gct 300960at tac ggag ggg gcg aag gag gtt gtg ggg cat gca gcg aag gat gct 100 3151008gga aag td gt ggg cat agg gg gt gtg ggg cat gca gcg aag gat gct 3101008gaa aag tg gtg gcd acg gcg agt aag gct gta aag agg aag 3051006gaa aag tg gtg gcd acg gcg agt agg ct gtg ggg cat gca gcg aag gaa gct 3101008gga aag tt gtg gcd acg gcg agg agg tt gtg ggg cat gca gcg aag gaa acc 3251006cgt gt tat act gg go cat gca gcg aag agg gaa ct 3251077cgt gt tac ggg gcd acg gcg agt aag gct gta aag agg aag gga act 3251077cat cat gga goc atg gca ga ag gct gta aag agg aag gga act 3251077cgt gt tac ggg gcd acg gcg gag aag gcg tgt gt ggg ga acg 3301077cgt gt			Gly	-				Ser				-	Leu	-			624
Leu Thr Gin Ala Gin Leu Leu Gin Phe Thr Ser Val Val Val Tyr Thr 225 230 236 236 Giy Cys Thr Giy Tyr Thr His Tyr Tyr His Thr Lys His Giy Ala Asp 246 250 250 250 250 250 Gag aca cag cct agt ta agt ag acg tat tat tct tgt tgt gag gtg cag Giu Thr Gin Pro Ser Leu Giy Thr Tyr Tyr Phe Cys Cys Giy Val Gin 260 265 270 Gig ctt at tg aag aag aca aag ta gga agg aag ga ag ag ag ag ag Arg gag atg ggt aat at gag gat cat tg tus Phe Ser Lie Phe Tyr Lys 275 280 285 Cga tcc tat tcg aag aag aac aag tca gga gga aag gat ag ca aag aag Arg Ser Tyr Ser Lys Lys Aan Lys Ser Giy Giy Lys Asp Ser Lys Lys 270 300 aat gat gat ggt at aat gag gat cat tg cag ag ag aag gat ag ca aag aag Arg Ser Tyr Ser Lys Lys Aan Lys Ser Giy Giy Lys Asp Ser Lys Lys 290 20 20 aat gat gat ggt at aat gag gat cat tg cag ag ag gat ag ca ag gat 300 300 at a tgg ag ggt gcg aag aga gag ggt gt gg gg cat gca agg ag gg aat 1008 110 Ser Giu Giy Ala Lys Giu Val Val Giy His Ala Ala Lys Asp Ala 325 326 2212 707 2212 707 2210 SEQ ID N0 60 2212 50 2212 707 2213 CRGNNISM: Thaleselosira preudonana 2400 > SEQUENCE: 60 Met Cys Ser Ser Pro Pro Ser Gin Ser Lys Thr Thr Ser Leu Leu Ala 1 5 Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Tyr Cys 20 20 20 20 20 20 20 21 20 22 20 23 24 24 25 20 25 20 25 20 20 25 20 20 20 20 20 20 20 20 20 20		Tyr					Leu					Pro					672
GIY Çiy ciys Thr GIY Tyr Thr His Thr Lyš His GIY XIA Åsp gag aca cag cct agt tta gga acg tat tat ttc tgt tg gga gtg cag 816 glu Thr GIN Pro Ser Leu GIY Thr Tyr Tyr Phe Cys Cys GIY Val GIN 816 gtg ttt gag atg gtt agt ttg ttt gta ctc ttt tcc atc ttt tat aaa 864 Val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser IIe Phe Tyr Lys 864 val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser IIe Phe Tyr Lys 912 grg ser Tyr Ser Lys Lys Asn Lys Ser GIY GIY Lys Asp Ser Lys Lys 950 Agn Asp App GIY Ann Ann GIU Asp GIN Cys His Ala Met Lys Asp 950 300 310 315 311 Ser GIY GIY Yis Ala Met Lys Asp 950 305 310 310 310 Asg gg gt cag ag gg gt cag ag gat gct 1008 11e Ser Glu GIY Ala Lys Glu Val Val GIY His Ala Ala Lys Asp Ala 320 326 330 335 335 gga aag ttg gtg gct acg gcg agt agt gat gct 1008 1077 345 345 350 350 1077 347 348 350 1077 347 348 109 1077 347 345 350 348	Leu	Thr				Leu					Ser					Thr	720
Glu Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln 260 260 270 261 Val Cln 270 gtg tit gag atg gtt agt tig tit gta ctc tit tcc atc tit tat aaa 220 864 280 285 864 val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys 285 285 912 864 grg ser Tyr Ser Lys Lys Aan Lys Ser Gly Gly Lys Aap Ser Lys Lys 198 912 912 960 aat gat gat agg aac aag gag gat caa tgt cac aag gct atg aag gat agc aag gat gct aan Aap Aap Gly Aan Am Glu Aap Gln Cys His Lys Aan Lys Aap Ala 325 960 305 ata tcg gag ggt gcg aag gag gtt gtg ggg cat gca gcg aag gat gct 1008 1008 315 335 1068 gga aag ttg gtg gct acg gcg agt aag gct gcg aag gaa gga act gcg gcg aag gaa gcg act 335 1056 1077 1077 grg val Thr Gly Ala Lys Glu Val Val Cly His Ala Val Lys Arg Lys Gly Thr 340 1056 1077 1077 grg val Thr Gly Ala Met 325 1077 1077 1077 1077 strop Ser Di No 60 101 1077 1077 1077 c2120 > SEQ ID No 60 10 15 10 15 c2120 > TPE: PRT 10 15 15 16 Arg Tyr Thr Thr Ala Ala Leu Leu Leu Thr Leu Thr Thr Thr Cys 30		-	-		Tyr					His	-	-			Ala	-	768
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Arg Ser Tyr Ser Lys Lys Am Lys Ser Gly Gly Lys Asp Ser Lys Lys 300960aat gat gat gag gat aat gag gat caa tgt cac ag gct atg aag gag Sin Glu Asp Glu Cys His Lys Ala Met Lys Asp 310960ata tcg gag ggt gcg aag gag gtt gtg ggg cat gca gcg gag gat gct 11e Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala 3251008gga aag ttg gtg gcd acg gcg agt aag gct gta aag agg aag gag aag 3401056Gly Lys Leu Val Ala Thr Ala Ser Lys Ala Val Lys Arg Lys Gly Thr 3401056cqt gtt act ggt gcc atg tag Asp Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala 101077c410> SEQUENCE: 6010Met Cys Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala 115Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Cys 2030His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu 4045Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu 5050Ser Lue Pro Ser Luys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys			Glu					Phe					Ile				864
Aen Asp Asp Gly Asn Aen Glu Asp Gln Cys His Lys Ala Met Lys Asp 310Set Lys Asp 320ata tog gag ggt gog aag gag gtt gtg ggg cat goa gog aag gat got 11e Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala 3251008gga aag ttg gtg got acg gog agt aag got gta aag agg aag gga act Gly Lys Leu Val Ala Thr Ala Ser Lys Ala Val Lys Arg Lys Gly Thr 3401056cgt gtt act ggt goc atg tag Asp Val Thr Gly Ala Met 3551077c210> SEQ ID N0 60 <211> LENGTH: 358 <212> TYPE: PRT <213> ORGNISM: Thalassiosira pseudonana1077c400> SEQUENCE: 6010Met Cys Ser Ser Pro Pro Ser Gln Ser Lys Thr Thr Ser Leu Leu Ala 115Arg Tyr Thr Thr Ala Ala Leu Leu Leu Leu Thr Leu Thr Thr Trp Cys 2025His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu 404045Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu 50Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asm Tyr Leu Ala Lys Lys		Ser					Asn					Lys					912
Ile Ser Glu Gly Ala Lys Glu Val Val Gly His Ala Ala Lys Asp Ala 325 330 330 335 335 335 335 335 335 335 33	Asn	Asp	-			Asn		-		-	His	-	-	-	-	Āsp	960
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20 25 30 His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu 35 35 Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu 50 55 Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys	Met				Pro	Pro	Ser	Gln	Ser	-	Thr	Thr	Ser	Leu		Ala	
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Glu	Gly	Arg 35	Met	Thr	Asn	Val	Glu 40	Thr	Met	Leu	Ala	Ile 45	Glu	Суз	Gly		

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Lys Pro Phe Glu Le 65	eu Lys Ser Phe Lys Leu Ala His 70 75	s Asn Leu Phe Leu 80
Phe Val Leu Ser Al 85	a Tyr Met Cys Leu Glu Thr Val 90	l Arg Gln Ala Tyr 95
Leu Ala Gly Tyr Se 100	er Val Phe Gly Asn Asp Met Glu 105	ı Lys Gly Ser Glu 110
Pro His Ala His Gl 115	y Met Ala Gln Ile Val Trp Ile. 120	e Phe Tyr Val Ser 125
Lys Ala Tyr Glu Ph 130	ne Val Asp Thr Leu Ile Met Ile 135 140	
Phe Asn Gln Val Se 145	er Val Leu His Val Tyr His His 150 155	s Ala Thr Ile Phe 160
Ala Ile Trp Phe Me 16	et Ile Ala Lys Tyr Ala Pro Gly 55 170	y Gly Asp Ala Tyr 175
Phe Ser Val Ile Le 180	eu Asn Ser Phe Val His Thr Val 185	l Met Tyr Ala Tyr 190
Tyr Phe Phe Ser Se 195	er Gln Gly Phe Gly Phe Val Lys 200	s Pro Ile Lys Pro 205
Tyr Ile Thr Ser Le 210	eu Gln Met Thr Gln Phe Met Ala 215 220	
Ser Leu Tyr Asp Ty 225	r Leu Tyr Pro Cys Asp Tyr Pro 230 235	o Gln Gly Leu Val 240
Lys Leu Leu Gly Va 24	ul Tyr Met Leu Thr Leu Leu Ala 15 250	a Leu Phe Gly Asn 255
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	ac gcg cgc gag tgg atc ggt gcg pp Ala Arg Glu Trp Ile Gly Ala 40	
	g acg acg atg tac ctg ttg ttc .a Thr Thr Met Tyr Leu Leu Phe 55 60	
	g aag cgc gag gcg ttc gac ccg a Lys Arg Glu Ala Phe Asp Pro 70 75	
	g tat cag acg gcg ttc aac gtc a Tyr Gln Thr Ala Phe Asn Val ; 90	

303

304

atg ttc gcg cg. Met Phe Ala Arg 100	g Glu Ile Ser				336
acc atg ccg tgg Thr Met Pro Trj 115					384
tgg ttg cac ta Trp Leu His Ty: 130					432
atg gtt gcg cg Met Val Ala Arg 145					480
cat cac gcc cty His His Ala Lev					528
gcc acg aac ga Ala Thr Asn Asj 18	p Cys Ile Asp				576
ttc att cac at Phe Ile His Il 195					624
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tgc ccg gtc ac Cys Pro Val Th					768
ctc gtg ctc tt Leu Val Leu Ph 260	e Gly Asn Phe				816
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Ile Asp Asn Va 35	l Asp Ala Arg	Glu Trp Ile 40	Gly Ala Leu 45	Ser Leu Arg	
Leu Pro Ala Il 50	e Ala Thr Thr 55	Met Tyr Leu	Leu Phe Cys 60	Leu Val Gly	
Pro Arg Leu Me [.] 65	t Ala Lys Arg 70	Glu Ala Phe	Asp Pro Lys 75	Gly Phe Met 80	
Leu Ala Tyr As	n Ala Tyr Gln 85	Thr Ala Phe 90	Asn Val Val	Val Leu Gly 95	

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Met	Phe	Ala	Arg 100	Glu	Ile	Ser	Gly	Leu 105	Gly	Gln	Pro	Val	Trp 110	Gly	Ser		
Fhr	Met	Pro 115	Trp	Ser	Asp	Arg	Lys 120	Ser	Phe	Lys	Ile	Leu 125	Leu	Gly	Val		
Trp	Leu 130	His	Tyr	Asn	Asn	Gln 135	Tyr	Leu	Glu	Leu	Leu 140	Asp	Thr	Val	Phe		
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His	His	Ala	Leu	Leu 165	Ile	Trp	Ala	Trp	Trp 170	Leu	Val	Суз	His	Leu 175	Met		
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Phe	Ile	His 195	Ile	Val	Met	Tyr	Ser 200	Tyr	Tyr	Leu	Met	Ser 205	Ala	Leu	Gly		
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Phe 225	Val	Ile	Val	Phe	Ala 230	His	Ala	Val	Phe	Val 235	Leu	Arg	Gln	Lys	His 240		
Суз	Pro	Val	Thr	Leu 245	Pro	Trp	Ala	Gln	Met 250	Phe	Val	Met	Thr	Asn 255	Met		
Leu	Val	Leu	Phe 260	Gly	Asn	Phe	Tyr	Leu 265	Lys	Ala	Tyr	Ser	Asn 270	Lys	Ser		
Arg	Gly	Asp 275	Gly	Ala	Ser	Ser	Val 280	Lys	Pro	Ala	Glu	Thr 285	Thr	Arg	Ala		
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atg	agt	ggc	tta	cgt			aac Asn									48	
1		-		5					10		-		-	15	-	0.5	
							gtc Val									96	
							agt Ser 40									144	
	-					-	gtg Val		-		-				-	192	
	-		-				cgt Arg				-		-	-		240	
	-						aga Arg	-				-				288	
ttc																336	

307

				tc aag gcc acg ne Lys Ala Thr 25	384
	eu Ala Leu			ta agt aaa ata al Ser Lys Ile	432
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		Ile Tyr His H		tt tcc ttt att le Ser Phe Ile 175	528
Trp Trp Ile I				ct tac ttc agc la Tyr Phe Ser 190	576
			Cys Met Tyr Th	cc tat tat cta nr Tyr Tyr Leu 05	624
	eu Ile Gly			cc aac tac ctt er Asn Tyr Leu	672
				ag ttt ttc ttc In Phe Phe Phe 240	720
		Tyr Cys Ala S		cg tat ccc aag nr Tyr Pro Lys 255	768
Phe Leu Ser L				tt ctc ggc ttg eu Leu Gly Leu 270	816
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Gln Trp Asp I 35	le Gly Pro	Val Ser Ser S 40	Ser Thr Ala H: 45	is Leu Pro Ala 5	
Ile Glu Ser P 50		Leu Val Thr S 55	Ser Leu Leu Pł 60	ne Tyr Leu Val	
Thr Val Phe L 65	eu Trp Tyr 70	Gly Arg Leu I	Thr Arg Ser Se 75	er Asp Lys Lys 80	
Ile Arg Glu P	ro Thr Trp 85		Phe Ile Ile Cy 90	ys His Asn Ala 95	
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Ala Tyr Gln A 115	sn Gly Tyr	Thr Leu Trp G 120	-	ne Lys Ala Thr 25	

Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys 245 250 Phe Leu Ser Lys Ile Leu Leu Val Tyr Met Met Ser Leu Leu Gly Leu Phe Gly His Phe Tyr Tyr Ser Lys His Ile Ala Ala Ala Lys Leu Gln Lys Lys Gln Gln <210> SEQ ID NO 71 <211> LENGTH: 1362 <212> TYPE: DNA <213> ORGANISM: Primula farinosa <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(1362) <223> OTHER INFORMATION: Delta-6 desaturase <400> SEQUENCE: 71 atg gct aac aaa tct cca cca aac ccc aaa aca ggt tac ata acc agc Met Ala Asn Lys Ser Pro Pro Asn Pro Lys Thr Gly Tyr Ile Thr Ser tca gac ctg aaa tcc cac aac aag gca ggt gac cta tgg ata tca atc Ser Asp Leu Lys Ser His Asn Lys Ala Gly Asp Leu Trp Ile Ser Ile cac ggc caa gtc tac gac gtg tcc tct tgg gcc gcc ctt cat ccg ggg His Gly Gln Val Tyr Asp Val Ser Ser Trp Ala Ala Leu His Pro Gly gge act gee eet ete atg gee ett gea gga eae gae gtg ace gat get Gly Thr Ala Pro Leu Met Ala Leu Ala Gly His Asp Val Thr Asp Ala tte etc geg tae eat ecc ect tee act gee egt etc etc ect ect etc Phe Leu Ala Tyr His Pro Pro Ser Thr Ala Arg Leu Leu Pro Pro Leu tet ace aac ete ett ett eaa aac eae tee gte tee eee ace tee tea Ser Thr Asn Leu Leu Gln Asn His Ser Val Ser Pro Thr Ser Ser gac tac cgc aaa ctc ctc gac aac ttc cat aaa cat ggc ctt ttc cgc Asp Tyr Arg Lys Leu Leu Asp Asn Phe His Lys His Gly Leu Phe Arg gcc agg ggc cac act gct tac gcc acc ttc gtc ttc atg ata gcg atg Ala Arg Gly His Thr Ala Tyr Ala Thr Phe Val Phe Met Ile Ala Met ttt cta atg agc gtg act gga gtc ctt tgc agc gac agt gcg tgg gtc

Phe	Leu 130	Met	Ser	Val	Thr	Gly 135	Val	Leu	Суз	Ser	Asp 140	Ser	Ala	Trp	Val	
	~	-	~	~~	~ ~	gca Ala	-	~~~		-				~	~~	480
				-		glà aaa										528
						atc Ile										576
						tgg Trp										624
						ccc Pro 215										672
						aac Asn										720
						gtg Val										768
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						ata Ile										1008
						gtg Val										1056
						aca Thr										1104
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						cag Gln										1200
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315

355 360 365 App Trop Phe His Gly Gly Leu Gln Phe Gln Val Glu His His Leu Phe 370 385 Fro Arg Met Pro Arg Gly Gln Phe Arg Lye Ile Ser Pro Phe Val Arg 400 Asp Trop Phe His Gly Gly Din Phe Arg Lye Ile Ser Pro Phe Val Arg 395 Arg Met Pro Arg Gly Gln Phe Arg Lye Ile Ser Pro Phe Val Arg 400 Asp Leu Cys Lye Lye We His Asn Leu Pro Tyr Ann Ile Ala Ser Phe Thr 405 Ile Ala Ser Phe Thr 405 Lye Ala Asn Val Phe Thr Leu Lye Thr Leu Arg Asn Thr Ala Ile Glu 435 Ile Glu Ala Ala Arg Ang Leu Ser Asn Pro Leu Pro Lye Asn Met Val Trp Glu Ala 445 Ile Clu Ala Calor SEQ ID NO 73 Clus Asn Met Val Trp Glu Ala Ala Arg Ang Phe User Asn Pro Leu Pro Lye Asn Met Val Trp Glu Ala Ala Yr Ang Phe Ibr Leu Lye Thr 435 Clus Asn Met Val Trp Glu Ala Calor SEQ ID NO 73 Clus TrpE: IDM Calor SEQ DENCE: 73 Clus Chron Trp Inte Thr Ser 10 The Thr Ser Ile 10 Pro Pro Pro Asn Pro Ipw Thr Gly Tyr Ile Thr Ser 10 16 Cas Gg Gg Gg Gg Ca Ca Ga Ca Ca Ca Ca Ca Cag Gg Gg Ct Ca Cg Gg Ct Ca Cag Gg Gg I Ca Ca Cag Agg Gg I Ca Ca Ga Ca Cag Gg Gg Ca Ca Gg Gg Ca Ca Cg Agg Aga 120 (Just Tyr App Val Ser Fr Phala Gly Ala Thr App Ala 15 122 Cas Gg Gg Gg Gg Ca Ca Ca Ga Ga Ca Ca Ga Ca
370 375 380 Pro Arg Met Pro Arg GLY GLN Phe Arg Lyg Ile Ser Pro Phe Val Arg 400 Acp Leu Cyg Lyg Lyg His Ann Leu Pro Tyr Ann Ile Ala Ser Phe Thr 415 Lyg Ala Aon Val Phe Thr Leu Lyg Thr Leu Arg Aon Thr Ala Ile Glu 420 Ala Arg App Leu Ser Ann Pro Leu Pro Lyg Ann Met Val Trp Glu Ala 440 420 Ala Arg App Leu Ser Ann Pro Leu Pro Lyg Ann Met Val Trp Glu Ala 445 421 420 421 420 421 420 421 420 421 420 421 422 422 421 422 421 422 421 422 422 421 422 422 422 422 422 422 423 424 424 425 425 426 427 428 429 429
385 390 395 400 Amp Leu Cys Lys Lyg His Amn Leu Pro Tyr Am Ile Ala Ser Phe Thr 405 410 Ala Ser Phe Thr 415 Lys Ala Asn Val Phe Thr Leu Lyg Thr 420 Leu Arg Asn Thr Ala Ile Glu 430 410 Ala Arg Asp Leu Ser Asn Pro Leu Pro Lys Asn Net Val Trp Glu Ala 435 Ala Arg Asp Leu Ser Asn Pro Leu Pro Lys Asn Net Val Trp Glu Ala 435 -210> SEQ ID NO 73 <211> LENOTH: 1862
405 410 415 Lyø Ala Aon Val Phe Thr Leu Lyø Thr Leu Arg Aon Thr Ala Ile Glu 420 420 Ala Arg Agp Leu Ser Aon Pro Leu Pro Lyø Aon Met Val Trp Glu Ala 435 430 Ala Arg Agp Leu Gly 450 440 -210> SEQ ID NO 73 <211> LENGTH: 1362 <212> CONTORS: <222> LOCATION: (1)(1362) <222> LOCATION: (1)(1362) <222> LOCATION: (1)(1362) <222> LOCATION: (1)(1362) <222> LOCATION: (1)(1362) <222> CONTRENTHERENANTION: Delta-6 desaturase <400> SEQUENCE: 73 atg gct aac aaa atc ccc aca acc acc aaa acca ggt tac att acc agc Ser Asp Leu Lyø Gly His Aon Lyø Ser Ser Trp Ala Gly App Leu Trp Ile Ser Ile 20 96 cac ggg gag gt tac gac gtg tc tc gc gg gc cg cc tc acc ccg ggg 315 144 gc ctg aaa ggg Ccc aac aaa cca cac gac gac gac dat acc gac gct 21 192 30 cac ggg gag gt tac gac gtg cc tc tc gc ag ga cac gac gta acc gac gct 315 192 30 cac ggg cag ta cac cat ct ct ct ct acc gc gc gc ct ct cc cc ct cc ct ct 55 240 50 gc at gcc cc cc tc tt gc cc tc tc acc gc gc ct ct cc cc ct ct ct ct cc cc ct ct ct
420425430Ala Arg Aep Leu Ser Asn Pro Leu Pro Lys Asn Met Val Trp Glu Ala 445445445440446445440446445447440448446448446450580 Th Leu Gly 450450580 Th No 73 (2112 TYPE INA (212) FEATORE: (222) FEATORE: (222) FEATORE: (222) FEATORE: (222) FEATORE: (222) FORTORE: (222) FORTORE: (223) FORTORE: (223) FORTORE: (223) FORTORE: (223) FORTORE: (223) FORTORE: (223) FORTORE: (224) FOR AND LYS AND FOR LYS THE GLY THE THE SET THE (227) FOR AND LYS AND LY
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As a get ac aaa tot cca cca ac cca ac cca aaa ac ggt tac att acc agc for pro Asn ProGly Tyr 11e Thr Ser 1548Met Ala Asn Lys Ser Pro Pro Asn ProLys Thr Gly Tyr 11e Thr Ser 10961Ser Asp Leu Lys Gly His Asn Lys Ala Gly Asp Leu Trp 11e Ser 11e 2096cac ggg gag gta tac gac gtg tot tot cg tgg gc gg cg ctt cac ccg ggg 40144His Gly Glu Val Tyr Asp Val Ser Ser Trp Ala Gly Leu His Pro Gly 45144ggc agt gcc ccc ta tg gc ctc gca gga cac gac gta acc gac gct 50192ggc agt gcc ccc ta tg gcc tt cac ccc ccc ccc 50240Y Ser Ala Pro Leu Met Ala Leu Ala Gly His Asp Val Thr Asp Ala 50192ctc ac acc ctc ct ct ct ct acc gcc cgc ctc ct ccc ctc ccc ctc 70240tt cta gcg tat cat cct cct tot acc gcc cgc ctc ccc ccc ccc ctc 70240tt cta gcg tat cat cct cct ct ca aac cac tc cg tc cc cc ccc ctc ccc ctc 85288ser Thr Asn Leu Leu Gln Asn His 100Ser Val Ser Pro Thr Ser Ser 90gca agg ggc cac act gct acc tc cc ct cc cac aac ttc gt at agt at gtg at 100gca agg ggc cac act gct acc gca gg tc ctt tg cac agt at at gtg at 115gca agg ggc cac act gcd ga gt cc tt tg acc agt agt at gtg at 115gca agg ggc cac act gcg ag tc ctt tg acc agt agt at gtg at 115gca agg ggc cac act ccc gga gt cc ctt gca acc gtg agt cc 100gca agg ggc cac act ggg gt cc ctt tg acc agt agt agt gg at 115gca agg ggc cac act gcg agt cc tt tg acc agt agt agt fc 115gca agg ggc cac act gga gt cc tt tg acc agt agt fc 115gca agg ggc cac act fc tac acc acc ttc fc 115gca agg ggc cac act fc <br< td=""></br<>
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Ala Arg Gly His Thr Ala Tyr Ala Thr Phe Val Ile Met Ile Val Met 115 115 ttt cta acg agc gtg acc gga gtc ctt tgc agc gac agt gcg tgg gtc 432 Phe Leu Thr Ser Val Thr Gly Val Leu Cys Ser Asp Ser Ala Trp Val 140 130 135 140 cat ctg gct agc ggc gca gca atg ggg ttc gcc tgg atc cag tgc gga 480 His Leu Ala Ser Gly Ala Ala Met Gly Phe Ala Trp Ile Gln Cys Gly 160 ttgg ata ggt cac gac tct ggg cat tac cgg att atg tct gac agg aaa 528 Trp Ile Gly His Asp Ser Gly His Tyr 170 175 175 tgg aac tgg ttc gcg cag gtc ctg agc aca aac tgc ctc cag ggg atc 576 576 trp Asn Trp Phe Ala Gln Val Leu Ser Thr Asn Cys Leu Gln Gly Ile 576
Phe Leu Thr Ser Val Thr Gly Val Leu Cys Ser Asp Ser Ala Trp Val 130 135 140 cat ctg gct agc ggc gca gca atg ggg ttc gcc tgg atc cag tgc gga 480 His Leu Ala Ser Gly Ala Ala Met Gly Phe Ala Trp Ile Gln Cys Gly 160 145 150 155 160 tgg ata ggt cac gac tct ggg cat tac cgg att atg tct gac agg aaa 528 Trp Ile Gly His Asp Ser Gly His Tyr Arg Ile Met Ser Asp Arg Lys 175 165 170 175 tgg aac tgg ttc gcg cag gtc ctg agc aca aac tgc ctc cag ggg atc 576 Trp Asn Trp Phe Ala Gln Val Leu Ser Thr Asn Cys Leu Gln Gly Ile 576
His Leu Ala Ser Gly Ala Ala Met Gly Phe Ala Trp Ile Gln Cys Gly 145 150 155 160 tgg ata ggt cac gac tct ggg cat tac cgg att atg tct gac agg aaa 528 Trp Ile Gly His Asp Ser Gly His Tyr Arg Ile Met Ser Asp Arg Lys 165 170 175 576 tgg aac tgg ttc gcg cag gtc ctg agc aca aac tgc ctc cag ggg atc 576 Trp Asn Trp Phe Ala Gln Val Leu Ser Thr Asn Cys Leu Gln Gly Ile
Trp Ile Gly His Asp Ser Gly His Tyr Arg Ile Met Ser Asp Arg Lys 165 170 tgg aac tgg ttc gcg cag gtc ctg agc aca aac tgc ctc cag ggg atc 576 Trp Asn Trp Phe Ala Gln Val Leu Ser Thr Asn Cys Leu Gln Gly Ile 576
Trp Asn Trp Phe Ala Gln Val Leu Ser Thr Asn Cys Leu Gln Gly Ile

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					nula	via:	lii									
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Met 1	мιа	ASN	гЛа	Ser 5	FLO	Pro	ASN	Pro	Lуз 10	Inr	сту	ıyr	тте	Thr 15	ьer	

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Ala	Arg	Gly 115	His	Thr	Ala	Tyr	Ala 120	Thr	Phe	Val	Ile	Met 125	Ile	Val	Met
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Trp	Ile	Gly	His	Asp 165	Ser	Gly	His	Tyr	Arg 170	Ile	Met	Ser	Asp	Arg 175	Lya
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Lys	Leu	Asn	Phe	Asp 245	Gly	Val	Ser	Arg	Phe 250	Leu	Val	Суз	Tyr	Gln 255	His
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Gln	Glu 290	Ile	Phe	Gly	Leu	Ala 295	Val	Phe	Trp	Val	Trp 300	Phe	Pro	Leu	Leu
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Ser	Tyr	Ser	Val	Thr 325	Gly	Ile	Gln	His	Val 330	Gln	Phe	Ser	Leu	Asn 335	His
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Lys	Lys	Gln 355	Thr	Ala	Gly	Thr	Leu 360	Asn	Ile	Ser	СЛа	Pro 365	Ala	Trp	Met
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Pro 385	Arg	Met	Pro	Arg	Gly 390	Gln	Phe	Arg	Гла	Ile 395	Ser	Pro	Phe	Val	Arg 400
Asp	Leu	Cys	Lys	Lys 405	His	Asn	Leu	Pro	Tyr 410	Asn	Ile	Ala	Ser	Phe 415	Thr
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	-	-	-		-	-				-	-	caa Gln	-			672	
	-		-				-				-	cgt Arg	-	-		720	
tgc	ccg	gtc	acc	ctt	cct	tgg	gcg	caa	atg	ttc	gtc	atg	acg	aac	atg	768	

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Leu	Pro 50	Ala	Ile	Ala	Thr	Thr 55	Met	Tyr	Leu	Leu	Phe 60	Суз	Leu	Val	Gly			
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His	His	Ala	Leu	Leu 165	Ile	-	Ala	_	170		Val	-		Leu 175	Met			
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Phe	Ile	His 195	Ile	Val	Met	Tyr	Ser 200	Tyr	Tyr	Leu	Met	Ser 205	Ala	Leu	Gly			
Ile	Arg 210	Суз	Pro	Trp	ГЛа	Arg 215	Tyr	Ile	Thr	Gln	Ala 220	Gln	Met	Leu	Gln			
Phe 225	Val	Ile	Val	Phe	Ala 230	His	Ala	Val	Phe	Val 235	Leu	Arg	Gln	Lys	His 240			
Сүв	Pro	Val	Thr	Leu 245	Pro	Trp	Ala	Gln	Met 250	Phe	Val	Met	Thr	Asn 255	Met			
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Arg	Gly	Asp 275	Gly	Ala	Ser	Ser	Val 280	ГЛа	Pro	Ala	Glu	Thr 285	Thr	Arg	Ala			
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			g ttc tgc ctg gtc u Phe Cys Leu Val 60	
			c ccg aag ggg ttc p Pro Lys Gly Phe	
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	g Glu Ile Ser		g ccc gtg tgg ggg n Pro Val Trp Gly 110	
			g atc ctc ctc ggg s Ile Leu Leu Gly 125	
			a ttg gac act gtg u Leu Asp Thr Val 140	
			c ttc ttg cac gtt r Phe Leu His Val 5	
			g gtg tgt cac ttg u Val Cys His Leu 175	
	p Cys Ile Asp		c gcg gcg tgc aac y Ala Ala Cys Asn 190	
	e Val Met Tyr	-	c atg tcg gcg ctc u Met Ser Ala Leu 205	
			g gct caa atg ctc n Ala Gln Met Leu 220	
			g ctg cgt cag aag l Leu Arg Gln Lys 5	
			c gtc atg acg aac e Val Met Thr Asn 255	
			g tac tcg aac aag a Tyr Ser Asn Lys	

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	_	35		Asp		-	40	_		-		45			_
Leu	Pro 50	Ala	Ile	Ala	Thr	Thr 55	Met	Tyr	Leu	Leu	Phe 60	Сүз	Leu	Val	Gly
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		-		Ala 85	-				90					95	-
			100	Glu			-	105	-				110	-	
		115	-	Ser	-	-	120			-		125		-	
_	130		-	Asn		135	-				140	-			
145			-	Lys	150		-			155					160
				Leu 165 Cys		-		-	170			-		175	
			180	Val		-		185		-			190		
		195		Trp			200					205			
	210	-		Phe	-	215	-				220				
225				Leu	230					235		-			240
-				245 Gly		-			250					255	
			260	-			-	265	-		-		270	-	
-	-	275	-	Ala			280	-				285	1111	лц	лıа
Pro	Ser 290	Val	Arg	Arg	Thr	Arg 295	Ser	Arg	Lys	Ile	Asp 300				

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				gag Glu												336
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				aac Asn												432
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				ttg Leu 165												528
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	-		-	ttc Phe			-				-	-	-	-		720
-	-	-		ctt Leu 245					-		-	-	-		-	768
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-		-		gcg Ala	-					-			-	-		864

	-		-	-	-	-		cga			-	taa				903		
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le A	ab	Asn 35	Val	Asp	Ala	Arg	Glu 40	Trp	Ile	Gly	Ala	Leu 45	Ser	Leu	Arg			
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eu A	la	Tyr	Asn	Ala 85	Tyr	Gln	Thr	Ala	Phe 90	Asn	Val	Val	Val	Leu 95	Gly			
∍t P	he	Ala	Arg 100	Glu	Ile	Ser	Gly	Leu 105	Gly	Gln	Pro	Val	Trp 110	Gly	Ser			
nr M	let	Pro 115	Trp	Ser	Asp	Arg	Lys 120	Ser	Phe	Lys	Ile	Leu 125	Leu	Gly	Val			
-	eu .30	His	Tyr	Asn	Asn	Gln 135	Tyr	Leu	Glu	Leu	Leu 140	Asp	Thr	Val	Phe			
et V 45	'al	Ala	Arg	Lys	Lys 150	Thr	Lys	Gln	Leu	Ser 155	Phe	Leu	His	Val	Tyr 160			
is H	lis	Ala	Leu	Leu 165	Ile	Trp	Ala	Trp	Trp 170	Leu	Val	Сүз	His	Leu 175	Met			
la T	'hr	Asn	Asp 180	Суз	Ile	Asp	Ala	Tyr 185	Phe	Gly	Ala	Ala	Cys 190	Asn	Ser			
he I	le	His 195	Ile	Val	Met	Tyr	Ser 200	Tyr	Tyr	Leu	Met	Ser 205	Ala	Leu	Gly			
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ys P	ro	Val	Thr	Leu 245	Pro	Trp	Ala	Gln	Met 250	Phe	Val	Met	Thr	Asn 255	Met			
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							tgg Trp						
							tac Tyr						
							atg Met						
							cac His						
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							gtg Val						
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337	
331	

338

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					ccg Pro											384		
					gaa Glu											432		
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					atc Ile											576		
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205 of 290

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339

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Phe	Leu	Thr	Gly		Ser	Met	Tyr	Met		Thr	Glu	Cys	Ala	Arg	Gln		
۵la	Tur	Leu	Glv	85 Glv	Tur	Lva	Leu	Dhe	90 Glv	Agn	Pro	Met	Glu	95 Lys	Glv		
nia	1 7 1	Бси	100		1 y 1	Цуб	Deu	105		11011	110	nee	110	цур	dry		
Thr	Glu	Ser 115	His	Ala	Pro	Gly	Met 120	Ala	Asn	Ile	Ile	Tyr 125	Ile	Phe	Tyr		
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Arg			Ile	Thr	Thr				Thr	Gln			Ala	Met	Val		
Val	210 Gln	50	V~1	Ψ * • ~	2 ~~~	215 Tyr	The end-	2~~	Dre	(*••~	220 Agn	T T	Dro	<i>c</i> 1~	Dro		
vai 225	GTU	ser	vai	ıyr	Asp 230		ıyr	ASU	51.0	Cys 235		ıyr	L.T.O	Gln	240		
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His	Phe	Ala 35	Phe	Pro	Ala	Ala	Thr 40	Ala	Thr	Pro	Gly	Leu 45	Thr	Ala	Glu		
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met	ніз	ser	ıyr	гда	va⊥	Pro	ьeu	σтλ	ьeu	Thr	va⊥	Рne	TYr	Leu	ьeu		

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								tcc Ser					864
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Ser 65	Leu	Pro	Ser	Leu	Lys 70	Tyr	Val	Thr	Asb	Asn 75	Tyr	Leu	Ala	Lys	Lys 80	
Tyr	Aab	Met	Lys	Ser 85	Leu	Leu	Thr	Glu	Ser 90	Met	Val	Leu	Tyr	Asn 95	Val	
Ala	Gln	Val	Leu 100	Leu	Asn	Gly	Trp	Thr 105	Val	Tyr	Ala	Ile	Val 110	Aab	Ala	
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ГÀа	Met	Asp	Gln	Val 165	Ser	Phe	Leu	His	Ile 170	Tyr	His	His	Thr	Thr 175	Ile	
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Leu 225	Thr	Gln	Ala	Gln	Leu 230	Leu	Gln	Phe	Thr	Ser 235	Val	Val	Val	Tyr	Thr 240	
Gly	Cys	Thr	Gly	Tyr 245	Thr	His	Tyr	Tyr	His 250	Thr	Lys	His	Gly	Ala 255	Asp	
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Arg	Ser 290	Tyr	Ser	Lys	Lys	Asn 295	Lys	Ser	Gly	Gly	Lys 300	Asp	Ser	Lys	Lys	
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Ile	Ser	Glu	Gly	Ala 325	Lys	Glu	Val	Val	Gly 330	His	Ala	Ala	Lys	Asp 335	Ala	
Gly	ГЛа	Leu	Val 340	Ala	Thr	Ala	Ser	Lys 345	Ala	Val	ГЛа	Arg	Lys 350	Gly	Thr	
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			-			acc Thr				-	-	-			-	768
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	-		-	-		aac Asn			-		-					864

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- 34	• /

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		275					280					285				
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	-	-			gag Glu 310			-	-	-	-					960
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	Ser	Leu	Pro 20		Asp	Суз	Phe	Glu 25		Ser	Val	Pro	Leu 30		Leu	
Tyr	Tyr	Thr 35	Val	Arg	Суз	Leu	Val 40	Ile	Ala	Val	Ala	Leu 45	Thr	Phe	Gly	
Leu	Asn 50	Tyr	Ala	Arg	Ala	Leu 55	Pro	Glu	Val	Glu	Ser 60	Phe	Trp	Ala	Leu	
Asp 65	Ala	Ala	Leu	Cys	Thr 70	Gly	Tyr	Ile	Leu	Leu 75	Gln	Gly	Ile	Val	Phe 80	
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Arg	Tyr	His	Leu 100	Leu	Asn	Phe	Val	Val 105	Gly	Thr	Phe	Met	His 110	Ser	Leu	
Ile	Leu	Thr 115	Pro	Phe	Glu	Ser	Trp 120	Lys	Leu	Thr	His	Arg 125	His	His	His	
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145		-	-		Pro 150			0		155					160	
		-		165	Tyr Phe				170				-	175		
			180		Leu			185			-		190			
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225					230 Glu	-				235					240	
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350

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Tyr	Val 50	Val	Ile	Glu	Gly	Val 55	Glu	Tyr	Asp	Val	Thr 60	Asp	Phe	Lys	His
Pro 65	Gly	Gly	Thr	Val	Ile 70	Phe	Tyr	Ala	Leu	Ser 75	Asn	Thr	Gly	Ala	Asp 80
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ГЛа	Ala	Leu	Ala 100	Ala	Leu	Pro	Ser	Arg 105	Pro	Ala	Lys	Thr	Ala 110	Lys	Val
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Phe 145	Ala	Glu	Leu	Ala	Ala 150	Met	Tyr	Ala	Leu	Gly 155	Thr	Tyr	Leu	Met	Tyr 160
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Pro	Ala	Val	Ala	Phe 245	Phe	Asn	Thr	Ala	Val 250	Glu	Asp	Asn	Arg	Pro 255	Arg
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His	Thr 370	Ile	Aab	Ile	Aab	Pro 375	Ser	Gln	Gly	Trp	Val 380	Asn	Trp	Leu	Met
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Thr	Leu	Asp	Ala	85 Lys	Asn	Arg	Val	Asn	90 Leu	Gly	Trp	Gly	Glu	95 Ala	Gln	
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		equei			1101	. De	ica-	s ue	sacu.	Lape						
_		-			-		-		-		-	-	-	cag Gln 15	-	48
gtg			-	ctg	-		-	-	ctg	-	-		-	gag Glu	-	96
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	aag Lys															480	
~ ~	aac Asn															528	
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Val	Ala	Leu	Tyr	Val 85	Leu	Gly	Гла	Val	Leu 90	His	Phe	Gly	Leu	Leu 95	Leu		
Gly	Val	Pro	Ala 100	Tyr	Leu	His	Gly	Leu 105	Ser	Asn	Ala	Ile	Val 110	Pro	Phe		
Leu	Ala	Tyr 115	Gly	Ala	Phe	Gly	Ser 120	Phe	Val	Leu	Суз	Trp 125	Phe	Phe	Ile		
Val	Ser 130	His	Asn	Leu	Glu	Ala 135	Leu	Thr	Pro	Val	Asn 140	Leu	Asn	Lys	Ser		
Thr 145	Lys	Asn	Asp	Trp	Gly 150	Ala	Trp	Gln	Ile	Glu 155	Thr	Ser	Ala	Ser	Trp 160		
Gly	Asn	Ala	Phe	Trp 165	Ser	Phe	Phe	Ser	Gly 170	Gly	Leu	Asn	Leu	Gln 175	Ile		
Glu	His	His	Leu 180	Phe	Pro	Gly	Met	Ala 185	His	Asn	Leu	Tyr	Pro 190	Lys	Met		
Val	Pro	Ile 195	Ile	LÀa	Asp	Glu	Сув 200	Ala	Lys	Ala	Gly	Val 205	Arg	Tyr	Thr		
Gly	Tyr	Gly	Gly	Tyr	Thr	Gly	Leu	Leu	Pro	Ile	Thr	Arg	Asp	Met	Phe		

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	01.0					015					0.0.0				
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Ser 225	Tyr	Leu	His	Lys	Суз 230	Gly	Arg	Thr	Ala	Lys 235	Leu	Ala			
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		-	-	-	-	gcc Ala		-				-			288
						acc Thr									336
	-		-			ttc Phe	-	-					-		384
-			<u> </u>		00	cga Arg 135	0						<u> </u>		432
						gcg Ala									480
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						aat Asn									624
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						cct Pro									720
						cga Arg									768

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	atc Ile														864
	cac His 290														912
	atg Met		-	-		-	-	-			-				960
	cgc Arg														1008
-	gcc Ala		-		-		-	-	-		-	-	-		1056
	ggc Gly														1104
	ctc Leu 370														1152
	cac His														1200
	caa Gln														1248
	gag Glu														1296
	tgg Trp														1344
	ggt Gly 450														1392
	ttt Phe									•			<u> </u>		1440
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<213> ORGANISM: Ostreococcus tauri

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Arg Ser Arg Va 35	l Arg Lys Thi	r Thr Glu 40	Arg Ser	Leu Ala 45	Arg Val	Arg
Arg Ser Thr Se 50	r Glu Lys Gly 55	/ Ser Ala	Leu Val	Leu Glu 60	Arg Glu	Ser
Glu Arg Glu Ly 65	s Glu Glu Gly 70	y Gly Lys	Ala Arg 75	Ala Glu	Gly Leu	Arg 80
Phe Gln Arg Pr	o Asp Val Ala 85	a Ala Pro	Gly Gly 90	Ala Asp	Pro Trp 95	Asn
Asp Glu Lys Tr 10		r Lys Trp 105	Thr Val	Phe Arg	Asp Val 110	Ala
Tyr Asp Leu As 115	p Pro Phe Phe	e Ala Arg 120	His Pro	Gly Gly 125	Asp Trp	Leu
Leu Asn Leu Al 130	a Val Gly Arg 135		Thr Ala	Leu Ile 140	Glu Ser	Tyr
His Leu Arg Pr 145	o Glu Val Ala 150	a Thr Ala	Arg Phe 155	Arg Met	Leu Pro	Lys 160
Leu Glu Asp Ph	e Pro Val Glu 165	ı Ala Val	Pro Lys 170	Ser Pro	Arg Pro 175	Asn
Asp Ser Pro Le 18		n Ile Arg 185	Asn Arg	Val Arg	Glu Glu 190	Leu
Phe Pro Glu Gl 195	u Gly Lys Asr	n Met His 200	Arg Gln	Gly Gly 205	Asp His	Gly
Asp Gly Asp As 210	p Ser Gly Phe 215		Leu Leu	Leu Met 220	Pro Cys	Thr
Tyr Ser Leu Pr 225	o Gly Val Pro 230	> Phe Arg	Leu Pro 235	Pro Arg	Val Ser	Arg 240
Gly Arg Gly Le	u Val Ser Arg 245	g Phe Arg	His Cys 250	Ala Asn	His Gly 255	Ala
Met Ser Pro Se 26		l Asn Gly 265	Val Leu	Gly Leu	Thr Asn 270	Asp
Leu Ile Gly Gl 275	y Ser Ser Leu	1 Met Trp 280	Arg Tyr	His His 285	Gln Val	Ser
His His Ile Hi 290	s Cys Asn Asr 295		Met Asp	Gln Asp 300	Val Tyr	Thr
Ala Met Pro Le 305	u Leu Arg Phe 310	e Asp Ala	Arg Arg 315	Pro Lys	Ser Trp	Tyr 320
His Arg Phe Gl	n Gln Trp Tyı 325	r Met Phe	Leu Ala 330	Phe Pro	Leu Leu 335	Gln
Val Ala Phe Gl 34		o Ile Ala 345	Ala Leu	Phe Thr	Arg Asp 350	Thr
Glu Gly Ala Ly 355	s Leu His Gly	7 Ala Thr 360	Thr Trp	Glu Leu 365	Thr Thr	Val
Val Leu Gly Ly 370	s Ile Val His 375	-	Leu Leu	Leu Gly 380	Pro Leu	Met
Asn His Ala Va 385	l Ser Ser Val 390	l Leu Leu	Gly Ile 395	Val Gly	Phe Met	Ala 400
Cys Gln Gly Il	e Val Leu Ala	a Cys Thr	Phe Ala	Val Ser	His Asn	Val

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Ala	Glu	Ala	Lys 420	Ile	Pro	Glu	Asp	Thr 425	Gly	Gly	Glu	Ala	Trp 430	Glu	Arg	
Asp	Trp	Gly 435	Val	Gln	Gln	Leu	Val 440	Thr	Ser	Ala	Asp	Trp 445	Gly	Gly	Lys	
Ile	Gly 450	Asn	Phe	Phe	Thr	Gly 455	Gly	Leu	Asn	Leu	Gln 460	Val	Glu	His	His	
Leu 465	Phe	Pro	Ala	Ile	Cys 470	Phe	Val	His	Tyr	Pro 475	Asp	Ile	Ala	Гла	Ile 480	
Val	Lys	Glu	Glu	Ala 485	Ala	ГЛа	Leu	Asn	Ile 490	Pro	Tyr	Ala	Ser	Tyr 495	Arg	
Thr	Leu	Pro	Gly 500	Ile	Phe	Val	Gln	Phe 505	Trp	Arg	Phe	Met	Lys 510	Asp	Met	
Gly	Thr	Ala 515	Glu	Gln	Ile	Gly	Glu 520	Val	Pro	Leu	Pro	Lys 525	Ile	Pro	Asn	
Pro	Gln 530	Leu	Ala	Pro	Lys	Leu 535	Ala									
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-		-				aag Lys	-		-	-	-	-			-	336
						gcc Ala										384
						gca Ala 135										432
						atg Met										480
cac	att	gga	tcg	gct	ctc	ttg	ttg	gga	ttg	ttc	tgg	cag	cag	tgt	gga	528

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												0011	0 1 11				
His	Ile	Gly	Ser	Ala 165	Leu	Leu	Leu	Gly	Leu 170	Phe	Trp	Gln	Gln	Cys 175	Gly		
												aag Lys				576	
		-		-						-		atg Met 205	-			624	
												cat His				672	
												gat Asp				720	
-		-				-		-		-	-	gct Ala	-	-		768	
												aag Lys				816	
												ttg Leu 285				864	
												ctc Leu				912	
												ctt Leu				960	
												tgg Trp				1008	
												tcc Ser				1056	
												gca Ala 365				1104	
	-					-				-	-	acc Thr		-		1152	
												atc Ile				1200	
												ggt Gly				1248	
												aga Arg				1296	
		-		-						-	-	gag Glu 445				1344	
_				_	-	-		-				gaa Glu		-		1392	
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Lys His Ile Thr 35	Pro Asp Asp	Ala Trp Val 40	Val His Gln 45	Asn Lys Val
Tyr Asp Val Ser 50	Asn Trp Tyr 55	Asp His Pro	Gly Gly Ala 60	Val Val Phe
Thr His Ala Gly 65	Asp Asp Met 70	Thr Asp Ile	Phe Ala Ala 75	Phe His Ala 80
Gln Gly Ser Gln	Ala Met Met 85	Lys Lys Phe 90	Tyr Ile Gly	Asp Leu Ile 95
Pro Glu Ser Val 100	Glu His Lys	Asp Gln Arg 105	Gln Leu Asp	Phe Glu Lys 110
Gly Tyr Arg Asp 115	Leu Arg Ala	Lys Leu Val 120	Met Met Gly 125	Met Phe Lys
Ser Ser Lys Met 130	Tyr Tyr Ala 135	Tyr Lys Cys	Ser Phe Asn 140	Met Cys Met
Trp Leu Val Ala 145	Val Ala Met 150	Val Tyr Tyr	Ser Asp Ser 155	Leu Ala Met 160
His Ile Gly Ser	Ala Leu Leu 165	Leu Gly Leu 170	Phe Trp Gln	Gln Cys Gly 175
Trp Leu Ala His 180	Asp Phe Leu	His His Gln 185	Val Phe Lys	Gln Arg Lys 190
Tyr Gly Asp Leu 195	Val Gly Ile	Phe Trp Gly 200	Asp Leu Met 205	Gln Gly Phe
Ser Met Gln Trp 210	Trp Lys Asn 215	Lys His Asn	Gly His His 220	Ala Val Pro
Asn Leu His Asn 225	Ser Ser Leu 230	Asp Ser Gln	Asp Gly Asp 235	Pro Asp Ile 240
Asp Thr Met Pro	Leu Leu Ala 245	Trp Ser Leu 250	Lys Gln Ala	Gln Ser Phe 255
Arg Glu Ile Asn 260	Lys Gly Lys	Asp Ser Thr 265	Phe Val Lys	Tyr Ala Ile 270
Lys Phe Gln Ala 275	Phe Thr Tyr	Phe Pro Ile 280	Leu Leu Leu 285	Ala Arg Ile
Ser Trp Leu Asn 290	Glu Ser Phe 295	Lys Thr Ala	Phe Gly Leu 300	Gly Ala Ala
Ser Glu Asn Ala 305	Lys Leu Glu 310	Leu Glu Lys	Arg Gly Leu 315	Gln Tyr Pro 320
Leu Leu Glu Lys	Leu Gly Ile 325	Thr Leu His 330	Tyr Thr Trp	Met Phe Val 335
Leu Ser Ser Gly 340	Phe Gly Arg	Trp Ser Leu 345	Pro Tyr Ser	Ile Met Tyr 350
Phe Phe Thr Ala 355	Thr Cys Ser	Ser Gly Leu 360	Phe Leu Ala 365	Leu Val Phe

Gly Leu Gly His Asn Gly Met Ser Val Tyr Asp Ala Thr Thr Arg Pro Asp Phe Trp Gln Leu Gln Val Thr Thr Thr Arg Asn Ile Ile Gly Gly His Gly Ile Pro Gln Phe Phe Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu Phe Pro Met Met Pro Arg Asn Asn Ile Ala Lys Cys His Lys Leu Val Glu Ser Phe Cys Lys Glu Trp Gly Val Lys Tyr His Glu Ala Asp Met Trp Asp Gly Thr Val Glu Val Leu Gln His Leu Ser Lys Val Ser Asp Asp Phe Leu Val Glu Met Val Lys Asp Phe Pro Ala Met <210> SEQ ID NO 99 <211> LENGTH: 1431 <212> TYPE: DNA <213> ORGANISM: Thalassiosira pseudonana <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(1431) <223> OTHER INFORMATION: Delta-5 desaturase <400> SEOUENCE: 99 atg ccc ccc aac gcc gat atc tcc cgc atc cgc aac cgc atc ccc acc Met Pro Pro Asn Ala Asp Ile Ser Arg Ile Arg Asn Arg Ile Pro Thr aaa aca ggt acc gtt gcc tct gcc gac aac aac gac ccc gcc acc caa Lys Thr Gly Thr Val Ala Ser Ala Asp Asn Asn Asp Pro Ala Thr Gln tcc gtc cga acc ctc aaa tct ctc aag ggc aac gag gtc gtc atc aac Ser Val Arg Thr Leu Lys Ser Leu Lys Gly Asn Glu Val Val Ile Asn ggc aca att tat gac att gct gac ttt gtc cat cct gga gga gag gtt Gly Thr Ile Tyr Asp Ile Ala Asp Phe Val His Pro Gly Gly Glu Val gtc aag ttc ttt ggt ggg aat gat gtt act att cag tat aat atg att Val Lys Phe Phe Gly Gly Asn Asp Val Thr Ile Gln Tyr Asn Met Ile cat ccg tat cat acg ggg aaa cat ctg gag aag atg aag gct gtt gga His Pro Tyr His Thr Gly Lys His Leu Glu Lys Met Lys Ala Val Gly aag gtt gta gat tgg cag tcg gac tac aag ttc gac acc ccc ttt gaa Lys Val Val Asp Trp Gln Ser Asp Tyr Lys Phe Asp Thr Pro Phe Glu cga gag atc aaa tca gaa gtg ttc aag atc gta cgt cgc ggg cgt gag Arg Glu Ile Lys Ser Glu Val Phe Lys Ile Val Arg Arg Gly Arg Glu ttc ggc aca aca ggc tac ttc ctc cgt gcc ttt ttc tac atc gct ctc Phe Gly Thr Thr Gly Tyr Phe Leu Arg Ala Phe Phe Tyr Ile Ala Leu ttc ttc acc atg caa tac act ttc gcc aca tgc acc acc ttc acc acc Phe Phe Thr Met Gln Tyr Thr Phe Ala Thr Cys Thr Thr Phe Thr Thr tac gat cac tgg tat cag agt ggt gta ttc atc gca att gtg ttt ggt Tyr Asp His Trp Tyr Gln Ser Gly Val Phe Ile Ala Ile Val Phe Gly

375

						glà aaa										576
						ccc Pro										624
						aac Asn 215										672
						aac Asn										720
-	-	-		-		gca Ala			-						-	768
						agg Arg										816
						tcg Ser		-			-				-	864
						cag Gln 295										912
		-	· ·		~ ~	aag Lys		~		-						960
		-			-	agc Ser			-				-	-		1008
						atg Met										1056
	-				-	ttg Leu							-	-	-	1104
		-	0	<u> </u>		aaa Lys 375	-			<u> </u>				00		1152
						tcg Ser										1200
						aac Asn										1248
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Ser Va	l Arg Th 35	nr Leu	Lys	Ser	Leu 40	Lys	Gly	Asn	Glu	Val 45	Val	Ile	Asn
Gly Th 50	r Ile Ty	yr Asp	Ile	Ala 55	Asp	Phe	Val	His	Pro 60	Gly	Gly	Glu	Val
Val Ly 65	s Phe Pł	ne Gly	Gly 70	Asn	Asp	Val	Thr	Ile 75	Gln	Tyr	Asn	Met	Ile 80
His Pr	o Tyr H:	is Thr 85	Gly	Lys	His	Leu	Glu 90	Lys	Met	ГЛа	Ala	Val 95	Gly
Lys Va	l Val As 1(ap Trp 00	Gln	Ser	Asp	Tyr 105	ГЛа	Phe	Asp	Thr	Pro 110	Phe	Glu
Arg Gl	u Ile Ly 115	/s Ser	Glu	Val	Phe 120	Гла	Ile	Val	Arg	Arg 125	Gly	Arg	Glu
Phe Gl 13	y Thr Th O	nr Gly	Tyr	Phe 135	Leu	Arg	Ala	Phe	Phe 140	Tyr	Ile	Ala	Leu
Phe Ph 145	e Thr Me	et Gln	Tyr 150	Thr	Phe	Ala	Thr	Сув 155	Thr	Thr	Phe	Thr	Thr 160
Tyr As	p His Tı	rp Tyr 165		Ser	Gly	Val	Phe 170	Ile	Ala	Ile	Val	Phe 175	Gly
Ile Se	r Gln Al 18		Ile	Gly	Leu	Asn 185	Val	Gln	His	Asp	Ala 190	Asn	His
Gly Al	a Ala Se 195	er Lys	Arg	Pro	Trp 200	Val	Asn	Asp	Leu	Leu 205	Gly	Phe	Gly
Thr As 21	p Leu II 0	le Gly	Ser	Asn 215	Гүз	Trp	Asn	Trp	Met 220	Ala	Gln	His	Trp
Thr Hi 225	s His Al	la Tyr	Thr 230	Asn	His	Ser	Glu	Lys 235	Asp	Pro	Asp	Ser	Phe 240
Ser Se	r Glu Pı	ro Met 245		Ala	Phe	Asn	Asp 250	Tyr	Pro	Ile	Gly	His 255	Pro
Lys Ar	g Lys Ti 20		His	Arg	Phe	Gln 265	Gly	Gly	Tyr	Phe	Leu 270	Phe	Met
Leu Gl	y Leu Ty 275	yr Trp	Leu	Ser	Thr 280	Val	Phe	Asn	Pro	Gln 285	Phe	Ile	Asp
Leu Ar 29	g Gln Ai 0	rg Gly	Ala	Gln 295	Tyr	Val	Gly	Ile	Gln 300	Met	Glu	Asn	Asp
Phe Il 305	e Val Ly	/s Arg	Arg 310	Lys	Tyr	Ala	Val	Ala 315	Leu	Arg	Met	Met	Tyr 320
Ile Ty	r Leu As	sn Ile 325		Ser	Pro	Phe	Met 330	Asn	Asn	Gly	Leu	Ser 335	Trp
Ser Th	r Phe G 34	•	Ile	Met	Leu	Met 345	Gly	Ile	Ser	Glu	Ser 350	Leu	Thr
Leu Se	r Val Le 355	eu Phe	Ser	Leu	Ser 360	His	Asn	Phe	Ile	Asn 365	Ser	Asp	Arg
Asp Pr 37	o Thr Al 0	la Asp	Phe	Lys 375	Lys	Thr	Gly	Glu	Gln 380	Val	Сув	Trp	Phe
Lys Se	r Gln Va	al Glu	Thr	Ser	Ser	Thr	Tyr	Gly	Gly	Phe	Ile	Ser	Gly

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420 425 430 Val Cys Lys Lys His Gly Val Asn Tyr Ala Tyr Tyr Pro Trp Ile Gly 445 Gln Asn Leu Val Ser Thr Phe Lys Tyr Met His Arg Ala Gly Ser Gly 450 Ala Asn Trp Glu Leu Lys Pro Leu Ser Gly Ser Ala 460 465 470 475 <210> SEQ ID NO 101 475 <211> LENGTH: 1449 475 <212> TYPE: DNA 420 <213> ORGANISM: Thalassiosira pseudonana 222> LOCATION: (1)(1449) <222> LOCATION: (1)(1449) <223> OTHER INFORMATION: Delta-5 desaturase <400> SEQUENCE: 101 atg cca ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg cca ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg cca ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg cca ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg cca ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg cca ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg coa ccc aac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg coa ccc ac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg coa ccc ac gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg coa ccc ac cc gcc gag gtc aaa aac ctc cgt tca cgt tcc atc cca Atg coa ccc accc cc a	
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Met Pro Pro Asn Ala Glu Val Lys Asn Leu Arg Ser Arg Ser Ile Pro	
	48
acg aag aag too agt toa tog toa too aco gog aao gat gat cog got S Thr Lys Lys Ser Ser Ser Ser Ser Ser Thr Ala Asn Asp Asp Pro Ala 20 25 30	96
acc caa tcc acc tca cct gtg aac cga acc ctc aag tct ttg aat gga 14 Thr Gln Ser Thr Ser Pro Val Asn Arg Thr Leu Lys Ser Leu Asn Gly 35 40 45	144
aac gaa ata gct att gac ggt gtc atc tat gat att gat ggc ttt gtc 19 Asn Glu Ile Ala Ile Asp Gly Val Ile Tyr Asp Ile Asp Gly Phe Val 50 55 60	192
cat cct gga gga gag gtt att agc ttc ttt gga ggc aac gat gtg act 24 His Pro Gly Gly Glu Val Ile Ser Phe Phe Gly Gly Asn Asp Val Thr 65 70 75 80	240
gta cag tac aaa atg att cat ccg tat cat aat agt aag cat ctc gag 28 Val Gln Tyr Lys Met Ile His Pro Tyr His Asn Ser Lys His Leu Glu 85 90 95	288
aag atg aga gcc gtt gga aag att gca gac tac tcc aca gag tac aag 33 Lys Met Arg Ala Val Gly Lys Ile Ala Asp Tyr Ser Thr Glu Tyr Lys 100 105 110	336
ttc gac aca ccc ttt gaa cga gag atc aaa tcc gaa gtg ttc aaa atc 38 Phe Asp Thr Pro Phe Glu Arg Glu Ile Lys Ser Glu Val Phe Lys Ile 115 120 125	384
gtc cgt cga gga cgt gaa ttc ggt aca aca gga tat ttc ctc cgt gcc 43 Val Arg Arg Gly Arg Glu Phe Gly Thr Thr Gly Tyr Phe Leu Arg Ala 130 135 140	432
ttc ttc tac att gct ctc ttc ttc acc atg caa tac acc ttc gcc aca48Phe Phe Tyr Ile Ala Leu Phe Phe Thr Met Gln Tyr Thr Phe Ala Thr145150155160	480
tgc act acc ttc acc acc tac gat cat tgg tat caa agt ggt gta ttc 52 Cys Thr Thr Phe Thr Thr Tyr Asp His Trp Tyr Gln Ser Gly Val Phe 165 170 175	528
atc gcc att gtg ttt ggt atc tca caa gct ttc att ggg ttg aat gta 57 Ile Ala Ile Val Phe Gly Ile Ser Gln Ala Phe Ile Gly Leu Asn Val 180 185 190	576
caa cat gat gcc aat cac gga gct gct agc aaa cga cct tgg gtg aat 62 Gln His Asp Ala Asn His Gly Ala Ala Ser Lys Arg Pro Trp Val Asn	576

382

_	_	195	_	_	_	_	200	_	_	_	_	205	_	_	_	
	ctc Leu 210															672
	ttg Leu															720
	gat Asp															768
	ccc Pro															816
	tac Tyr															864
	cca Pro 290															912
	cag Gln															960
	ctt Leu															1008
	aat Asn															1056
	agc Ser															1104
	gaa Glu 370															1152
	caa Gln															1200
	ggt Gly			•	000	<u> </u>										1248
	cat His															1296
	ccg Pro															1344
	tat Tyr 450															1392
	ggg ggg															1440
	gcg Ala	tag														1449

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<212> TYPE: PRT <213> ORGANISM: Thalassiosira pseudonana

-continued

< 400)> SH	EQUEI	ICE :	102											
Met 1	Pro	Pro	Asn	Ala 5	Glu	Val	Гла	Asn	Leu 10	Arg	Ser	Arg	Ser	Ile 15	Pro
Thr	Lys	Lys	Ser 20	Ser	Ser	Ser	Ser	Ser 25	Thr	Ala	Asn	Asp	Asp 30	Pro	Ala
Thr	Gln	Ser 35	Thr	Ser	Pro	Val	Asn 40	Arg	Thr	Leu	Lys	Ser 45	Leu	Asn	Gly
Asn	Glu 50	Ile	Ala	Ile	Asp	Gly 55	Val	Ile	Tyr	Asp	Ile 60	Asp	Gly	Phe	Val
His 65	Pro	Gly	Gly	Glu	Val 70	Ile	Ser	Phe	Phe	Gly 75	Gly	Asn	Asp	Val	Thr 80
Val	Gln	Tyr	Lys	Met 85	Ile	His	Pro	Tyr	His 90	Asn	Ser	ГЛа	His	Leu 95	Glu
Lys	Met	Arg	Ala 100	Val	Gly	ГЛа	Ile	Ala 105	Asp	Tyr	Ser	Thr	Glu 110	Tyr	Lys
Phe	Asp	Thr 115	Pro	Phe	Glu	Arg	Glu 120	Ile	ГЛа	Ser	Glu	Val 125	Phe	Lys	Ile
Val	Arg 130	Arg	Gly	Arg	Glu	Phe 135	Gly	Thr	Thr	Gly	Tyr 140	Phe	Leu	Arg	Ala
Phe 145	Phe	Tyr	Ile	Ala	Leu 150	Phe	Phe	Thr	Met	Gln 155	Tyr	Thr	Phe	Ala	Thr 160
Суз	Thr	Thr	Phe	Thr 165	Thr	Tyr	Asp	His	Trp 170	Tyr	Gln	Ser	Gly	Val 175	Phe
Ile	Ala	Ile	Val 180	Phe	Gly	Ile	Ser	Gln 185	Ala	Phe	Ile	Gly	Leu 190	Asn	Val
Gln	His	Asp 195	Ala	Asn	His	Gly	Ala 200	Ala	Ser	Lys	Arg	Pro 205	Trp	Val	Asn
Asp	Leu 210	Leu	Gly	Ser	Gly	Ala 215	Asp	Leu	Ile	Gly	Gly 220	Суз	Lys	Trp	Asn
Trp 225	Leu	Ala	Gln	His	Trp 230	Thr	His	His	Ala	Tyr 235	Thr	Asn	His	Ala	Asp 240
Lys	Asp	Pro	Asp	Ser 245	Phe	Ser	Ser	Glu	Pro 250	Val	Phe	Asn	Phe	Asn 255	Asp
Tyr	Pro	Ile	Gly 260	His	Pro	Lys	Arg	Lys 265	Trp	Trp	His	Arg	Phe 270	Gln	Gly
Leu	Tyr	Phe 275	Leu	Ile	Met	Leu	Ser 280	Phe	Tyr	Trp	Val	Ser 285	Met	Val	Phe
Asn	Pro 290	Gln	Val	Ile	Asp	Leu 295	Arg	His	Ala	Gly	Ala 300	Ala	Tyr	Val	Gly
Phe 305	Gln	Met	Glu	Asn	Asp 310	Phe	Ile	Val	ГÀа	Arg 315	Arg	ГÀа	Tyr	Ala	Met 320
Ala	Leu	Arg	Ala	Met 325	Tyr	Phe	Tyr	Phe	Asn 330	Ile	Tyr	САа	Pro	Ile 335	Val
Asn	Asn	Gly	Leu 340	Thr	Trp	Ser	Thr	Val 345	Gly	Ile	Ile	Leu	Leu 350	Met	Gly
Val	Ser	Glu 355	Ser	Phe	Met	Leu	Ser 360	Gly	Leu	Phe	Val	Leu 365	Ser	His	Asn
Phe	Glu 370	Asn	Ser	Glu	Arg	Asp 375	Pro	Thr	Ser	Glu	Tyr 380	Arg	LÀa	Thr	Gly
Glu 385	Gln	Val	Суз	Trp	Phe 390	Lys	Ser	Gln	Val	Glu 395	Thr	Ser	Ser	Thr	Tyr 400
Gly	Gly	Ile	Val	Ala 405	Gly	Сүз	Leu	Thr	Gly 410	Gly	Leu	Asn	Phe	Gln 415	Val

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386

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435 440 445 Vyr Tyr Pro Tyr Ile Trp Gln Am Leu His Ser Thr Val Ser Tyr Met 450 450 470 470 450 470 475 470 470 475 470 470 475 470 470 475 470 475 470 475 470 475 470 475 470 475 470 475 470 475 470 475 470 475 470 475 470 470 475 470 470 475 470 470 475 470 470 475 470 471 10 470 1210 587 470 420 470 470 445 470 470 445 470 470 446 470 470 440 470 470 440 470 470 <td< td=""><td>Glu</td><td>His</td><td>His</td><td></td><td>Phe</td><td>Pro</td><td>Arg</td><td>Met</td><td></td><td>Ser</td><td>Ala</td><td>Trp</td><td>Tyr</td><td></td><td>Phe</td><td>Ile</td><td></td></td<>	Glu	His	His		Phe	Pro	Arg	Met		Ser	Ala	Trp	Tyr		Phe	Ile	
450 455 460 1450 177 Glu Leu Gin Pro Leu Ser Gly 465 470 475 480 115 170 475 480 1211: LENGTH: 1211: LENGTH: 1212 1212: TYPE: NAME/KEY: CES 1223: VOCATION: (1)(1)(1)(1): 1223: DOCATION: (1)(1)(1): 121: LENGTH: 15 1223: DOCATION: (1)(1)(1): 15 15 1223: DOCATION: (1)(1)(1): 15 15 1220: DEACTION: (1)(1)(1)(1): 15 15 15 10 15 15 15 16 10 11 15 15 15 10 20 25 144 15 15 16 10 20 25 144 15 15 16 110 110 110 110 114 16 192 110 20 25 16 16 192 192 110 110 15 16 192 <td< td=""><td>Ala</td><td>Pro</td><td>-</td><td>Val</td><td>Arg</td><td>Glu</td><td>Ile</td><td>-</td><td>Lys</td><td>Lys</td><td>His</td><td>Gly</td><td></td><td>Arg</td><td>Tyr</td><td>Ala</td><td></td></td<>	Ala	Pro	-	Val	Arg	Glu	Ile	-	Lys	Lys	His	Gly		Arg	Tyr	Ala	
165 170 175 180 111 Laborn H. 1512 Line Laborn H. 1512 Line Laborn H. 1512 112.11 Laborn H. 1512 Line Laborn H. 1512 Line Laborn H. 1512 112.11 Laborn H. 1512 Line Laborn H. 1512 Line Laborn H. 1512 112.11 Laborn H. 1512 Line Laborn H. 1512 Line Laborn H. 1512 112.11 Laborn H. 1512 Line Laborn H. 1512 Line Laborn H. 1512 122.23 LOCATION: (1). (1512) Line Laborn H. 1500 Line Laborn H. 1500 122.23 LOCATION: (1). (1512) Line Laborn H. 1500 Line Laborn H. 1500 122.23 Core aad cat cat cat gag cat cg ca cat gag cat cg ca cat cat agg cat cg cat cat cat agg cat cg cat cat cat agg cat cg cat cat cat agg cat cg agg cg cg cat cat cat agg cat cg agg cat cg cat cat cat agg cat cg agg cat cg cat cat agg cat cg cat cat agg cat cg cat cat agg cat cg cat	Tyr		Pro	Tyr	Ile	Trp		Asn	Leu	His	Ser		Val	Ser	Tyr	Met	
2210 - SEQ ID NO 103 2211 - LEMOTH: 1512 2222 JUCATION: CIL . (1512) 2223 - OTHER INFORMATION: Delta-4 desaturase 2200 - PENTURE: 2223 - OTHER INFORMATION: Delta-4 desaturase 2400 - SEQUENCE: 103 Attg tgc acc ggc acc ctc cca gca tcc acc gca cag ctc ang tcc acc 48 10 10 10 10 10 10 10 10 10 10 10 10 10 1	His 465	Gly	Thr	Gly	Thr		Ala	Arg	Trp	Glu		Gln	Pro	Leu	Ser		
<pre>2115 ENGUTH: 1512 2213 FUPE: DNA 2213 ORGANISM: Thalassionira pseudonana 2206 FEATURE: 2211 MAME/KEY: CDS 2223 IOCATION: (1)(1512) 2223 IOCATION: (1)(1512) 2223 IOCATION: (1)(1512) 2223 IOCATION: (1)(1512) 2223 IOCATION: (1)(1512) 2223 IOCATION: (1)(1512) 2223 IOCATION: (1)(1512) 223 IOCATION: (1)(1512) 224 IOCATION: (1)(1512) 225 IOCATION: (1)(1512) 225 IOCATION: (1)(1512) 226 IOCATION: (1)(1512) 226 IOCATION: (1)(1512) 227 IOCATION: (1)(1512) 228 IOCATION: (1)(1512) 229 IOCATION: (1)(1512) 220 IOCATION: (1)(1512) 221 IOCATION: (1)(1512) 222 IOCATION: (1)(1512) 222 IOCATION: (1)(1512) 223 IOCATION: (1)(1512) 223 IOCATION: (1)(1512) 224 IOCATION: (1)(1512) 225 IOCATION: (1)(1512) 226 IOCATION: (1)(1512) 227 IOCATION: (1)(1512) 228 IOCATION: (1)(1512) 229 IOCATION: (1)(1512) 220 IOCATION: (1)(1512) 221 IOCATION: (1)(1512) 222 IOCATION: (1)(1512) 222 IOCATION: (1)(1512) 223 IOCATION: (1)(1512) 224 IOCATION: (1)(1512) 225 IOCATION: (1)(1512) 226 IOCATION: (1)(1512) 227 IOCATION: (1)(1512) 228 IOCATION: (1)(1512) 228 IOCATION: (1)(1512) 229 IOCATION: (1)(1512) 220 IOCATI</pre>	Arg	Ala															
A A	<211 <212 <213 <220 <221 <222 <222 <223	1> LH 2> TY 3> OH 0> FH 1> NH 2> LG 3> OY	ENGTH (PE : RGAN) EATUH AME / I CAT: CHER	H: 1 DNA ISM: RE: KEY: ION: INF	512 Tha CDS (1) ORMA	(1	512)	-									
BerLysProClinGlinGlinHisGluHisArgThrIleSerLysSerGlu202025252622526262cactac14430303030303030302020accaccaccaccacctac144acuAlHisAnThrProLysSerAlaTrpCys4040SerAlaTrpCysAlaValValNa1925055SerAsnAsnAsnAsnAsnAsn1925055SerAsnAsnAsnAsnAsnAsnAsn5055SerAsnAsnAsnAsnAsnAsnAsn5055SerAsnAsnAsnAsnAsnAsnAsn5055SerAsnAsnAsnAsnAsnAsnAsn5055SerAsnAsnAsnAsnAsnAsnAsnAsn5055SerAsnAsnAsnAsnAsnAsnAsnAsnAsn5055SerAsnAsnAsnAsnAsnAsnAsnAsnAsnAsn51SerAsnAsnAsnAsnAsnAsnAsnAsn <td>atg</td> <td>tgc</td> <td>aac</td> <td>ggc</td> <td>aac Asn</td> <td></td> <td></td> <td></td> <td></td> <td>Thr</td> <td></td> <td></td> <td></td> <td></td> <td>Ser</td> <td></td> <td>48</td>	atg	tgc	aac	ggc	aac Asn					Thr					Ser		48
Ala Gin His Asn Thr Pro Lys Ser Åla Trp Cys Åla Val His Ser 45 His Ser 45 act coc goc acc gac coa toc cac toc acc toc acc aca aaa caa caa cac goa cac thr Pro Åla Thr Asp Pro Ser His Ser Asn Asn Lys Gin His Åla His 55 192 att acc gac att acc gac ttt gog toc cgc cat cca ggg gga gac teu Val Leu Asp Ile Thr Asp Phe Åla Ser Årg His Pro Gly Gly Asp 55 240 att acc toc gga att acc gga att acc gac ttt gog toc reg gtg tg tt gaa aca beu Val Leu Ala Ser Gly Lys Åsp Åla Ser Val Leu Phe Glu Thr 90 288 att acc cat cac dgg ggg to cog acg tot toc att caa aag ctg cag att 100 336 acc at cac a cac gtga gat cog acg tot ctc att caa aag ctg cag att 100 336 agg gtg atg gag gag gag gag gag gag gag				Gln					His					Lys			96
Thr Pro Åla Thr Åsp Pro Ser His Ser Asn Asn Lys Gln His Åla His 240 sta gtc ctc gac att acc gac ttt gcg tcc cgc cat cca ggg gga gac 240 seu Val Leu Asp Ile Thr Asp Phe Ala Ser Arg His Pro Gly Gly Asp 75 80 sta gtc ctc ctc gct tcc ggc aat gac gcc tcg gtg ctg ttt gaa aca 288 288 seu Ile Leu Leu Ala Ser Gly Lys Asp Ala Ser Val Leu Phe Glu Thr 95 336 seu Ile Leu Leu Ala Ser Gly Val Pro Thr Ser Leu Ile Gln Lys Leu Gln Ile 100 336 ryr His Pro Arg Gly Val Pro Thr Ser Leu Ile Gln Lys Leu Gln Ile 110 384 set tt gac ttt act gtg ttg aag agg agg ggg ttg gga cgg ttg Asp 240 384 shy Val Met Glu Glu Glu Ala Phe Arg Asp Ser Phe 125 Ser Trp Thr 125 sat tct gac ttt tat act gtg ttg aag agg agg ggg ttg gag cgg ttg 432 432 shy Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg 140 Val Glu Arg Leu 140 shy Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg 140 Val Glu Arg Leu 140 shy Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg 140 Val Glu Arg Leu 140 shy Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg 140 Val Glu Arg Leu 140 shy Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg Gly Ser Lys Glu Ile Trp Ile Lys 160 480 shu Glu Arg Gly Leu Asp Arg Arg Gly Ser Lys Cys Leu Tyr Lys Met Tyr 175 160 shy Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg Gly Ser Lys Cys Leu Tyr Lys Met Tyr 175 <td></td> <td></td> <td>Gln</td> <td></td> <td></td> <td></td> <td></td> <td>Lys</td> <td></td> <td></td> <td></td> <td></td> <td>Āla</td> <td></td> <td></td> <td></td> <td>144</td>			Gln					Lys					Āla				144
Leu Asp Ile Thr Asp Phe Ala Ser Arg His Pro GIY GY Asp 80255707580261ctc ctc ctc gct tcc ggc aaa gac gcc tcg gtg ctg ttt gaa aca 85288281Leu Ile Leu Ala Ser GIY Lys Asp Ala Ser Val Leu Phe Glu Thr 9095336282gag gtt ccg acg tct ctc att caa aag ctg cag att 100336336293ggg ggg ggg ggg ggg gg ct ccg acg tct ctc att caa aag ctg cag att 100100336294gag gag gag gag gcg ttt ccg gat tcg gat tcg ttt tac agt tgg act 120384394395Ser Phe Tyr Ser Trp Thr 125384394115120120120394120120120140395Ser Asp Phe Tyr Thr Val Leu Lys Arg Arg Val Val Glu Arg Leu 130432394394395Ser Lys Glu Ile Trp Ile Lys 160432394130150150155394150150155394150150395155157160396150155156397155156398155157399160160391161190393165155394160395155157395156156396150155397155398150399155158399150399150150399150<		Pro					Ser					Lys					192
JeuIleLeuLeuAlaSerGIVLysAspAlaSerValLeuPheGluThrStaccatccaccgggagttccgacgtctctcattcaaaagccgcagatt336Staccatccacdgggagglgglgglgttccgacgtctctcattcaaaagccgcagatt336Stacfloflofloflofloflofloflofloflofloflofloflogaggtggtggaggaggaggaggcgtttcgggatcggattfloflofloflogattctgacflogattctgacfloflofloflofloflofloflofloflogaggaggagggggggggaagggggggaggaflofloflogasflofloflofloflofloflofloflofloflogasgagggggggggaagggggggaaggggaagggga </td <td></td> <td></td> <td></td> <td></td> <td></td> <td>Thr</td> <td></td> <td></td> <td></td> <td></td> <td>Arg</td> <td></td> <td></td> <td></td> <td></td> <td>Āsp</td> <td>240</td>						Thr					Arg					Āsp	240
TyrHisProArgGlyValProThrSerLeuIleGlnLysLeuGlnIleJgaAtgAgggaggaggaggaggaggaggaggagftrher </td <td></td> <td></td> <td></td> <td></td> <td>Āla</td> <td></td> <td></td> <td></td> <td>-</td> <td>Āla</td> <td>-</td> <td></td> <td>-</td> <td></td> <td>Glu</td> <td></td> <td>288</td>					Āla				-	Āla	-		-		Glu		288
Sily Val Met Glu Glu Glu Glu Ala Phe Arg Asp Ser Phe Tyr Ser Trp Thr 115 115 120 125 115 117 121 gat tot gac ttt tat act gtg ttg aag agg agg ggt gga Cgg ttg 130 120 125 125 125 125 125 gat tot gac ttt tat act gtg ttg aag agg agg ggt Cg aag Arg Val Val Glu Arg Leu 130 130 135 140 140 140 140 gag gag agg ggg ttg gac agg agg gga tcg aaa gga att tgg atc aag 140 140 140 140 140 140 gat dag ggg ggt tg gac agg agg ggt tcg aaa gga att tgg atc aag 140 140 140 160 160 160 gat dag gg ggt tg gac agg tgg tgg tac tgg tac tgt ttg tac aag atg tat 150 155 155 160 160 gat ttg ttc ttg ttg gtt gga ttt tgg tac tgt ttg tac aag atg tat 175 175 160 177 gat teg proven 165 150 170 170 175 175 160 gat tac gt tag gat atc gat cag tac ggt att tag tac tgt ttg tac tac agg tat gcc att gcc tat tot att 177 175 175 175 175 act acg tcg gat atc gat cag tac ggt att agg tac tgg cag tac ttg gga acc ttt gcg gca ttc atc ggc acg tgt att caa cac gat 190				Arg					Ser					Leu			336
AspSerAspPheTyrThrValLeuLysArgArgValValGluArgLeu130135135135140140140140140140gaggagggggggttggacaggagggggaggggaatcaaggluGluArgGlyLeuAspArgGlySerLysGluIleTrpIleLys145150150150155160160160getttgttcttgttgggatttggttgttgttg145150150177TyrTyr175160528getttgttgttgttgttgttgttgttgttg528actacgtggatacgggtattggcattgg576actacgtggatacgggattgccattgcctattctattfhrThrSer185186190116AlaTyrSer190624ggaatgggaaccttdgcgaccttdfcgfcafcagaatgggaaccttdgcgaccttdfcafcafcafullhiatgggaaccttdgcgfcafca <td< td=""><td></td><td></td><td>Met</td><td></td><td></td><td></td><td></td><td>Phe</td><td></td><td></td><td></td><td></td><td>Tyr</td><td></td><td></td><td></td><td>384</td></td<>			Met					Phe					Tyr				384
Silu Glu Arg Gly Leu Asp Arg Arg Gly Ser Lys Glu Ile Trp Ile Lys 145 150 155 160 get ttg ttc ttg ttg ggt gga ttt tgg tac tgt ttg tac aag atg tat 528 Ala Leu Phe Leu Leu Val Gly Phe Trp Tyr Cys Leu Tyr Lys Met Tyr 165 170 175 576 act acg tcg gat atc gat cag tac ggt att gcc att gcc tat tct att 576 Thr Thr Ser Asp Ile Asp Gln Tyr Gly Ile Ala Ile Ala Tyr Ser Ile 180 185 190 190 624 gga atg gga acc ttt gcg gca ttc atc ggc acg tgt att caa cac gat 624 Sly Met Gly Thr Phe Ala Ala Phe Ile Gly Thr Cys Ile Gln His Asp		Ser					Val					Val					432
Ala Leu Phe Leu Val Gly Phe Trp Tyr Cys Leu Tyr Lys Met Tyr 165 170 175 act acg tcg gat atc gat cag tac ggt att gcc att gcc tat tct att 576 Thr Thr Ser Asp Ile Asp Gln Tyr Gly Ile Ala Ile Ala Tyr Ser Ile 180 185 190 gga atg gga acc ttt gcg gca ttc atc ggc acg tgt att caa cac gat 624 Gly Met Gly Thr Phe Ala Ala Phe Ile Gly Thr Cys Ile Gln His Asp						Asp					Lys					Lys	480
Thr Thr Ser Asp Ile Asp Gln Tyr Gly Ile Ala Ile Ala Tyr Ser Ile 180 185 190 gga atg gga acc ttt gcg gca ttc atc ggc acg tgt att caa cac gat 624 Gly Met Gly Thr Phe Ala Ala Phe Ile Gly Thr Cys Ile Gln His Asp	-	-		-	Leu	-				Tyr	-	-		-	Met		528
Gly Met Gly Thr Phe Ala Ala Phe Ile Gly Thr Cys Ile Gln His Asp				Asp					Gly					Tyr			576
		-	Gly				-	Phe			_	-	Ile			-	624

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													0 1 1 1	ucu		
								aac Asn								672
								gcg Ala								720
								tat Tyr								768
	~ ~						000	gag Glu 265	•	•	•		•			816
-	-	-		-	-	-		tcc Ser		-					-	864
								tgg Trp								912
								atg Met								960
								ttg Leu								1008
								ttt Phe 345								1056
								ttg Leu								1104
-		-	-		-	-		acg Thr		-	-		-			1152
-	-	-		-			-	ttg Leu		-		-				1200
								gta Val								1248
								tcc Ser 425								1296
								gga Gly				-				1344
								aca Thr								1392
								tac Tyr								1440
-		-		-	-			aag Lys	-		-		-	-		1488
-	ggt Gly		-	-	-	gag Glu	tag									1512

<210> SEQ ID NO 104 <211> LENGTH: 503

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Ser	Lys	Pro	Gln 20	Gln	Gln	His	Glu	His 25	Arg	Thr	Ile	Ser	Lys 30	Ser	Glu
Leu	Ala	Gln 35	His	Asn	Thr	Pro	Lys 40	Ser	Ala	Trp	САа	Ala 45	Val	His	Ser
Thr	Pro 50	Ala	Thr	Asp	Pro	Ser 55	His	Ser	Asn	Asn	Lys 60	Gln	His	Ala	His
Leu 65	Val	Leu	Asp	Ile	Thr 70	Asp	Phe	Ala	Ser	Arg 75	His	Pro	Gly	Gly	Asp 80
Leu	Ile	Leu	Leu	Ala 85	Ser	Gly	Lys	Asp	Ala 90	Ser	Val	Leu	Phe	Glu 95	Thr
Tyr	His	Pro	Arg 100	Gly	Val	Pro	Thr	Ser 105	Leu	Ile	Gln	Lys	Leu 110	Gln	Ile
Gly	Val	Met 115	Glu	Glu	Glu	Ala	Phe 120	Arg	Asp	Ser	Phe	Tyr 125	Ser	Trp	Thr
Asp	Ser 130	Aab	Phe	Tyr	Thr	Val 135	Leu	Lys	Arg	Arg	Val 140	Val	Glu	Arg	Leu
Glu 145	Glu	Arg	Gly	Leu	Asp 150	Arg	Arg	Gly	Ser	Lys 155	Glu	Ile	Trp	Ile	Lys 160
Ala	Leu	Phe	Leu	Leu 165	Val	Gly	Phe	Trp	Tyr 170	Суз	Leu	Tyr	Lys	Met 175	Tyr
Thr	Thr	Ser	Asp 180	Ile	Asp	Gln	Tyr	Gly 185	Ile	Ala	Ile	Ala	Tyr 190	Ser	Ile
Gly	Met	Gly 195	Thr	Phe	Ala	Ala	Phe 200	Ile	Gly	Thr	Суз	Ile 205	Gln	His	Asp
Gly	Asn 210	His	Gly	Ala	Phe	Ala 215	Gln	Asn	Lys	Leu	Leu 220	Asn	Lys	Leu	Ala
Gly 225	Trp	Thr	Leu	Asp	Met 230	Ile	Gly	Ala	Ser	Ala 235	Phe	Thr	Trp	Glu	Leu 240
Gln	His	Met	Leu	Gly 245	His	His	Pro	Tyr	Thr 250	Asn	Val	Leu	Asp	Gly 255	Val
Glu	Glu	Glu	Arg 260	Lys	Glu	Arg	Gly	Glu 265	Asp	Val	Ala	Leu	Glu 270	Glu	Lys
Asp	Gln	Asp 275	Phe	Glu	Val	Ala	Thr 280	Ser	Gly	Arg	Leu	Tyr 285	His	Ile	Asp
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His	Gly	Val	Leu	Arg 325	Gly	Val	Gly	Leu	Phe 330	Val	Ile	Gly	His	Leu 335	Ala
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Lys	Lys	Lys	Gly	Glu 405	Lys	Asn	Ser	Val	Pro 410	Ser	Val	Pro	Phe	Asn 415	Asp	
Trp	Ala	Ala	Val 420	Gln	Cys	Gln	Thr	Ser 425	Val	Asn	Trp	Ser	Pro 430	Gly	Ser	
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His	Leu 450	Phe	Pro	Ser	Ile	Cys 455	His	Thr	Asn	Tyr	Cys 460	His	Ile	Gln	Asp	
Val 465	Val	Glu	Ser	Thr	Cys 470	Ala	Glu	Tyr	Gly	Val 475	Pro	Tyr	Gln	Ser	Glu 480	
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	att Ile 130															432
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	aac Asn															528
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CSIRO Exhibit 1013

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Pro Pro Try Clin Net Set 118 Arg Lys Kis Les And 100 att													0011	C III,	aca			
Aon Hi B 11e Clu Lyd Xep Tyr Ser His Lyd Trp Tyr Ser Xig Aep Clu ttt gat gat ato cca caa cto tat aag aca ttt ggo tac aac cca aga 210 210 210 210 210 210 210 210 210 210	Pro	Phe	Val		Trp	Gln	Met	Ser		Arg	Lys	His	His		Asn	His		
Phe Asp Asp lie Pro Gln Leu Tyr Lys Thr Phe Gly Tyr Asn Pro Asg 210 225 225 225 225 226 226 227 227 227 227 227 227 227 227			Ile	-	-	-		Ser		-			Ser	-	-		624	
Met Bot Gin Leu Pro Phe Leu Tyr Phe Net Tyr Leu Àla Leu Gly He 225 ca gat ggt ggg cat gft gft ct tac gga aga atg tgg gaa gga gfg Pro App Gly Gly His Val Val Phe Tyr Gly Arg Met Trp Glu Gly Val 246 ca tig cag aag aag tit gat gct gct att tct gft gcc gta tca tgt Ser Leu Gin Lyr Lyr Phe App Ala Ala He Ser Val Ala Val Ser Cyr 270 gca act gct gga tcg ctt gg atg gat ggg aga gcg tca ca gga gra gf Gra act gct gga tcg ctt tgg atg at atg ggt gct act a tgt 226 vr Met Val Pro Trp Leu Val Lau Ser Trp Trp Leu Phe Met 225 rue Gra tig gat gct ct tgg at ggt ggt cct tc atg g Gra act gct gga tcg ct tgg at ggt gg cc tc datg g 120 vr Met Val Pro Trp Leu Val Leu Ser Trp Trp Leu Phe Met 290 rue Gra aga gag gct aca cat ca gaa gac gga ag ct atca ct gat 200 rue Met Gly For Trp Leu Val Leu Ser Trp Trp Leu Phe Met 290 rue Gra His Ser Glu App Gly Lyr Leu Tyr Tr App 201 Tr Trp He Ula His Ser Glu App Gly Lyr Leu Tyr Tr App 202 rue Gra tac ct ga cat cat ca gaa gga cg tc dg ac ggt tg gc 1008 rue Gra Trp Phe Tur Phe Tr 290 rue Glu His Ser Glu App Gly Lyr Jap Arg Ber Glu 315 rue Gga aag tt aca ttg gaa aag gg ag ct tc at gg ac ggt cg t 335 rue Gga aag ct tt gga aca gg ga cg t cac 335 rue Gga aag ct tg gt cc cac tg tt t tt gaa cg tg ta cct cac tag at gag 335 rue Gga cag gct cac tg tt ct tt gaa ag gga atg gaa ag ga cag aag 335 rue Gga cag gct cac tg tt ct tt ga acg tg ta cct cac ta ag at ag 335 rue Gga aag cct tg ga cag tg gaa ag ga ag ga ag ga ag 335 rue Gag atg acc Ga ag gct ctt gga aag gga ag ga ag ga ag ga ag ga ag 335 rue Cac tg tr t tt gaa acg cg ta cc cat ta cac ag at ta gag 335 rue Fin Fin Fin Glu Ja Ala Leu Val Lyr Gly Met App Glu Thr Gly Glu Lyr 336 rue Try Tyr Tyr Tyr Tyr Tyr Tyr Tyr Tyr Tyr		Āsp					Leu					Gly					672	
Pro Asp GIY GIY Hie Val Val Phe Tyr CIY ASG Met Trp OL GIY Val 255 ta ttg cag aag ag ttt gat get get att tet gtg gec gta tea tgt 816 Ser Leu GIN Lys Lys Phe Asp Ala Ala II e Ser Val Ala Val Ser Cys 864 gaa acg gt cg ggt teg ct ttgg at ga at atg ggt aca gca gac ttc acg 864 Ala Thr 71 Ala GIY Ser Leu Trp Met Asn Met GIY Thr Ala Asp Phe Thr 225 gtg gt tg atg gt cct ttgg cta gtt cta teg tgg tgg ctc ttc atg 912 gtg gt atgo atg gtt cct tag cat cat tea gaa gac gga aag cta tac act gat 960 305 717 Leu Gin Hie Hie Ser Glu Asp GIY Lys Leu Tyr Thr Asp Arg Ser 960 316 912 912 gaa acg ttt aca ttt gaa aag gga gga Cgt cac aca tg atg gat ga cg gd ac gf tcg 1008 306 917 Leu Gin Hie Hie Ser Glu App Glu Thr Val Asp Arg Ser 1008 306 918 940 940 919 918 940 941 920 921 921 1008 307 926 927 1008 308 926 927 1008 309 928 929 920 1014 300 929 920 1014 1029 310<	Met					Phe					Tyr		<u> </u>			Ile	720	
Ser Leu Gh, Lyg Lyg Phe Amp Ala Ala Ile Ser Val Ala Val Ser Cyg 270 gca act gct gga tog ctt tgg atg aat atg ggt aca gca gac ttc acg Ala Thr Ala Gly Ser Leu Trp Met Ann Met Gly Thr Ala App Phe Thr 275 864 gtg gta tgc atg gtt cct tgg cta gtt cta tcg tgg tgg ctc ttc atg 2912 912 gtd gta tgc atg gtt cct tgg cta gtt cta tcg tgg tgg ctc ttc atg 300 912 gta aca tac ctt cag cat cat tca gaa gac gga aag cta tac act gat 960 910 305 910 911 310 925 912 gaa acg ttt aca tt gaa aag gga gc ctc gg ac cgt tgg 1008 960 Glu Thr Phe Glu Lyg Gly Ala Phe Glu Thr Val App Arg Ser 330 935 326 925 9330 gaa acg ttt aca tt gaa aag gga gc tt ca cac tga tg ac ggt cac 1056 940 Tyr Gly Lyg Leu Ile Ann Arg Net Ser His His Met Met App Gly His 356 946 340 345 950 gca gct acc ga gct ctt gtg aag gat gg at gga acg gga cag aaa 1152 940 355 940 941 Pro His Tyr Arg Leu Glu 365 950 943 940 940 951 940 940 940 953 950 940 940 953 950 940 940 940		•		000	His					Gly					Gly		768	
Ala Thr Ala GI y Ser Leu Try Mei Asn Mei GI y Thr Ala Asp Phe Thr 275 280 gt gt t t t t t t t t t t t t t t t t t				Lys					Ala					Val			816	
Val Val Val Pro Try Leu Soft Try Leu Pro Try Soft 960 gta aca tac ct cag cat			Ala					Met					Ala				864	
Yal Thr Tyr Leu Gln His His Ser Glu Asp Gly Lys Leu Tyr Thr Asp 305 310 310 310 310 310 310 310 320 gaa acg ttt aca ttt gaa aag gga gc ttc gGU Thr Val Asp Arg Ser 325 1008 1008 1008 gaa acg ttt aca ttt gaa cag tg gg cc tc gGU Thr Val Asp Arg Ser 325 1056 1056 Tyr Gly Lys Leu Ile Asm Arg Met Ser His His Met Met Asp Gly His 340 356 1014 Yal Ya His His Leu Phe Phe Glu Arg Yal Pro His Tyr Arg Leu Glu 355 104 104 Yal Xa Sis 360 360 365 1104 Yal Xa His His Leu Phe Phe Glu Arg Yal Pro His Tyr Arg Leu Glu 370 1104 1104 Yal Xa His His Leu Phe Phe Glu Arg Yal Pro His Tyr Arg Leu Glu 375 1104 1104 Yal Ya His His Leu Yal Yay Gly Met Asp Glu Thr Gly Gln Lys 370 1152 1152 Grad cc gaa gct ctt gtg aaa gga atg gat gat acg cag atg gat gt gt 120 1200 1200 His Leu Tyr Lys Tyr Ti Le Asp Thr Pro Asp Phe Ann Ala Glu Ile Val 385 1200 1210 385 405 410 400 1257 210> SEQ ID NO 106 1257 1257 1257 211> OKGANISM: Thalassiosira pseudonana 10 15 1257		Val					Trp					Trp					912	
Glu Thr Phe Thr Phe Glu Lys Gly Ala Phe Glu Thr Val Asp Arg Ser 325 1056 tac ggc aag ttg atc aac cga atg tcg cat cac atg atg ggc ggt cac 1056 Tyr Gly Lys Leu Ile Aen Arg Met Ser His His Met Met Asp Gly His 340 1104 gtg gtg cac cac ttg ttc ttt gaa cgt gta cct cac tac aga tta gag 1104 Val Val His His Leu Phe Phe Glu Arg Val Pro His Tyr Arg Leu Glu 365 gca gct acc gaa gct ctt gtg aaa gga atg gat acg gga cag aaa 1152 Ala Ala Thr Glu Ala Leu Val Lys Gly Met Asp Glu Thr Gly Gln Lys 1200 370 375 380 aca gga ttg cac aac at ggt tc ctt gtt gaa ggg agg aca tac aaa 1200 ass full Lea Asp Thr Pro Asp Phe Asn Ala Glu Glu Glu Asn Tle Lys 1200 aac gga ttt cgc gac aat tgg ttc ctt gtt gaa gag gag aca tc aaa 1248 Asn Gly Phe Arg Asp Asn Trp Phe Leu Val Glu Glu Glu Asn Tle Lys 1257 agg gag tag 1257 Arg Glu 1257 c210> SEQ ID NO 106 1257 c211> SEQ ID NO 106 1257 c212> TYPE: PET 121 c213> ORGANISM: Thalassiosira pseudonana 1257 c400> SEQUENCE: 106 15 Met Tyr Arg Leu Thr Ser Thr Phe Leu Ile Ala Leu Ala Phe Ser Ser 11 15 Ser Ile A	Val				-	His			-	-	Gly	-				Asp	960	
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His Leu Tyr Lys Tyr Ile Asp Thr Pro Asp Phe Asn Ala Glu Ile Val 385 390 aac gga ttt cgc gac aat tgg ttc ctt gtt gaa gag gag aac atc aaa 1248 Asn Gly Phe Arg Asp Asn Trp Phe Leu Val Glu Glu Glu Glu Asn Ile Lys 1257 agg gag tag 1257 <210> SEQ ID NO 106 1257 <211> LENTH: 418 12257 <212> TYPE: PRT 123 <213> ORGANISM: Thalassiosira pseudonana 400 <400> SEQUENCE: 106 15 Met Tyr Arg Leu Thr Ser Thr Phe Leu Ile Ala Leu Ala Phe Ser Ser 15 Ser Ile Asn Ala Phe Ser Pro Gln Arg Pro Pro Arg Thr Ile Thr Lys 30 Ser Lys Val Gln Ser Thr Val Leu Pro Ile Pro Thr Lys Asp Asp Leu 10	-	Āla		-	-		Val			-	-	Glu	-		-		1152	
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Met Tyr Arg Leu Thr Ser Thr Phe Leu IIe Ala Leu Ala Phe Ser Ser 1 Ser Ile Asn Ala Phe Ser Pro Gln Arg Pro Pro Arg Thr IIe Thr Lys 20 Ser Lys Val Gln Ser Thr Val Leu Pro IIe Pro Thr Lys Asp Asp Leu	<21 <21	1> L1 2> T1	ENGTI YPE :	H: 43 PRT	18	lass	iosi:	ra p:	seudo	onana	a							
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20 25 30 Ser Lys Val Gln Ser Thr Val Leu Pro Ile Pro Thr Lys Asp Asp Leu		Tyr	Arg	Leu		Ser	Thr	Phe	Leu		Ala	Leu	Ala	Phe		Ser		
	Ser	Ile	Asn		Phe	Ser	Pro	Gln		Pro	Pro	Arg	Thr		Thr	Lys		
	Ser	ГÀЗ		Gln	Ser	Thr	Val		Pro	Ile	Pro	Thr	-	Asp	Asp	Leu		

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Val 65	Asn	Thr	Pro	Pro	Arg 70	Ala	Gly	Thr	Ile	Met 75	Lys	Met	Leu	Pro	Lys 80
Glu	Thr	Phe	Asn	Ile 85	Asp	Thr	Ala	Thr	Ser 90	Leu	Gly	Tyr	Phe	Gly 95	Met
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Thr	Ser	Asp 115	Gln	Tyr	His	Ala	Leu 120	Pro	Leu	Pro	Leu	Gln 125	Ala	Ala	Thr
Val	Ile 130	Pro	Phe	Gln	Leu	Leu 135	Ala	Gly	Phe	Ala	Met 140	Trp	Суз	Met	Trp
Cys 145	Ile	Gly	His	Asp	Ala 150	Gly	His	Ser	Thr	Val 155	Ser	Lys	Thr	Lys	Trp 160
Ile	Asn	Arg	Val	Val 165	Gly	Glu	Val	Ala	His 170	Ser	Val	Val	Суз	Leu 175	Thr
Pro	Phe	Val	Pro 180	Trp	Gln	Met	Ser	His 185	Arg	Lys	His	His	Leu 190	Asn	His
Asn	His	Ile 195	Glu	Lys	Asp	Tyr	Ser 200	His	Lys	Trp	Tyr	Ser 205	Arg	Asp	Glu
Phe	Asp 210	Asp	Ile	Pro	Gln	Leu 215	Tyr	Lys	Thr	Phe	Gly 220	Tyr	Asn	Pro	Arg
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Glu	Thr	Phe	Thr	Phe 325	Glu	Lys	Gly	Ala	Phe 330	Glu	Thr	Val	Aab	Arg 335	Ser
Tyr	Gly	Lys	Leu 340	Ile	Asn	Arg	Met	Ser 345	His	His	Met	Met	Asp 350	Gly	His
Val	Val	His 355	His	Leu	Phe	Phe	Glu 360	Arg	Val	Pro	His	Tyr 365	Arg	Leu	Glu
Ala	Ala 370	Thr	Glu	Ala	Leu	Val 375	Lys	Gly	Met	Asp	Glu 380	Thr	Gly	Gln	Lys
His 385	Leu	Tyr	Lys	Tyr	Ile 390	Asp	Thr	Pro	Asp	Phe 395	Asn	Ala	Glu	Ile	Val 400
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Arg Glu

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-		-			tct Ser		-	-							-	336	
					ctt Leu											384	
					aac Asn											432	
					ttt Phe 150											480	
	-			-	ctc Leu				-			-		-		528	
					tta Leu											576	
					tgg Trp											624	
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					gca Ala 230											720	
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-	50		-	-		55					60		Ala				
65 65	Cys	Trp	Val	Ala	A1a 70	H1S	GIU	Cys	GIY	нія 75	GIY	Ala	Phe	Ser	Asp 80		
Asn	Lys	Thr	Leu	Gln 85	Asp	Ala	Val	Gly	Tyr 90	Val	Leu	His	Ser	Leu 95	Leu		
			100			-		105					His 110				
		115					120					125	Ala Leu				
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			180					185					190 Asp				
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Tyr 305	Lys	Ala	Gln	Met	Ala 310	Thr	Asp	Ala	Leu	Lys 315	Glu	Ala	Tyr	Pro	Asp 320	
Leu	Tyr	Leu	Tyr	Asp 325	Pro	Thr	Pro	Ile	Ala 330	Thr	Ala	Thr	Trp	Arg 335	Val	
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Met 1	GIY	ГЛа	GIY	GIY 5	Arg	Ser	Val	Thr	Arg 10	Ala	GIn	Thr	Ala	GIU 15	ГЛа	
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					ааа Lув											144
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	-			-	ctc Leu 70	-	-		-	-	-		-	-	-	240
		-			acg Thr				-						-	288
					aaa Lys											336
					ctc Leu											384
			-		tct Ser			-	-			-				432
					gcg Ala 150											480
			-		cat His	-	-					-	-			528
-					atc Ile	-	_		-		-			-		576
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lie Tyr Leu Met Giy phe Åla Ser Thr Giy Arg Leu Giy Gin Arg Giy 240 ang gaa ctt cag get gga gag atc atc gac cat tac cgt cct tgg agt 1/25 aag gaa ctt cag get gga gag atc atc gac cat tac cgt cct tgg agt 255 aag gaa ctt cag get gga gag atc atc gac cat tac cgt cct tgg agt 255 aag gaa ctt cag get gga gag atc atc gac cat tac cgt cct tgg agt 255 aag atg ttc ccc acc aag ttg cga ttc aaa att get ctt tog aca ctt 1/27 gga gtg att gcc gcc tgg gtt ggg ttg tac ttt get gca caa gag tat 1/28 gag dtc ttg ccc gtg gtt ctt tag tac att gge cca ctc atg tgg aat 1/2 gag gct gg ct gtg ct gtg ctc tac act gg gtc aad gag cac ttg gca caa tag gg cc aa gag tag att gac gcc cg atg ggt agt gad tag gca atg gg atg atg arg and 70 N and 7		Phe					Ile					Val					672	
Lys Glu Leu Gln Äta Gly Glu Ile Ile Åep His Tyr Arg Pro Trp ser 255aag atg tt c cc acc aag ttg cga tt aaa att gct ctt tc g aca ctt Lys Met Phe Pro Thr Lys Leu Arg Phe Lys Ile Ala Leu Ser Thr Leu 260816gga gtg att gcc gcc tgg gtt ggg ttg tac ttt gct gca caa gag tat Gly Val Ile Ala Ala Trp Val Gly Leu Tyr Phe Ala Ala Gln Glu Tyr 275864gga gtg att gcc gcc tgg gtt ct gt gt ac att ggc ca ct atg tgg aat 275912gga gtg tt gc gcc gtg gtt ct tt gg tac att ggc ca ct atg tgg aat 275912gga gcg tgg ctt gtg ct ct ac act gg cc ag ca at gat ccc tcc Gln Ala Trp Leu Val Leu Tyr Thr Trp Leu Gln His Asn App Pro Ser 310912gtg cct caa tat gga agt gac gaa tgg aca tgg tca ag gga gt ttg Val Pro Gln Tyr Gly Ser App Glu Trp Thr Trp Val Lys Gly Ala Leu 3261008stg gca gt at gat cgc ccg tat ggt at ct tt gac ttc tc cat ca aag 3401006att gga agc act cac gta gct cat cat ttg ttc cac gag at gg cc atg 3351056att gga agc act cac gta gct cat cat ttg ttc cac gag at gc cat tt 11041104le Gly Ser Thr His Val Ala His His Leu Phe His Glu Met Pro Phe 3551104aag ggg dt gt gct act gcg tcg at ca ag ggt ttc tg gag cag 11521120agg gtg gc caag act tg cac tat at tg agg gat gt gg gc at gt gg 11521200agg gtg gc caag act tg cac tat at tg agg gat gt gg ga gt tcg 11521200aag ggg tcg gc agg act tg cac tat at tg agg agg gd gg gc cag 11521200aag ggg gt gt gc act gcg tag gc tag gg gg gc cag 11521200aag gg gc dt tac aac tat gat cca acg acg gt gg gc atg gg gg1201aag gg gc by gc asg act tg cat tat at gag gag gtg gc atg gg1202	Ile					Phe					Arg					Gly	720	
Lyō Met Phe Pro Thr Lyō Leù Arg Phe Lyg Ile Äla Leu Ser Thr Leu 200 266 270 266 270 270 270 280 267 280 270 280 280 270 280 280 280 280 290 280 280 280 280 280 280 280 280 280 28					Āla					Āsp					Trp		768	
Giv valI le Ala Ala Trp valGiv LeuTyr PreAla Ala GLn Glu Tyr 285gga gtt ttg ccc gtg gtt ctt tgg tac att ggc ca ct atg tgg aat 290912cag gcg tgg ctt gtg ctc tac act tgg ctt cag cac aat gat ccc tcc 310912cag gcg tgg ctt gtg ctc tac act tgg ctt cag cac aat gat ccc tcc 310960gdg gt ct gtg ctc tac act tgg ctt cag cac aat gat ccc tcc 310960gdg gc ct caa tat gga agt gac gaa tgg aca tgg gtc aag gga gct tg 3251008Val Pro Gln Tyr Giv Ser Asp Glu Trp Thr Trp Val Lys Giv Ala Leu 3251008ser Thr Ile Asp Arg Pro Tyr Giv Ile Phe App Phe Phe His His Lys 3401056ser Thr Ile Asp Arg Pro Tyr Giv Ile Phe App Phe Phe His His Lys 3401104Ile Giv Ser Thr His Val Ala His His Leu Phe His Giu Met Pro Phe 3551104370375380aag gcg gat gtg gct act gg gt ca cag gd gt tc tt gg gc cag 3701152370375380aag gg act tac aat ta gat cca acg cct tgg tat gtg gca atg gg 3801200370375380aag gg gt gc caag att gt cat tat ti gag gat gtg gat agg 4001200385390270380390aag gg ag at tgg agg agt gg agg tgg gat gg 4201200385390aag gg ag ag agg agg agg agg agg agg 420385390386390387390388390389391385390390392391393392393393393				Pro					Phe					Ser			816	
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ValProGinTyrGlySerAspGluTipThrTipValLysGlyAlaLeu 335tcgacgattgatcccccgttdgatactttdaca	Gln					Leu					Gln			-		Ser	960	
Ser Thr Ile App Arg Pro Tyr Gly Ile Phe Asp Phe Phe His His Lys 340Ilo 4 350att gga agc act cac gta gct cat cat ttg ttc cac gag atg cca ttt Ile Gly Ser Thr His Val Ala His His Leu Phe His Glu Met Pro Phe 3551104atc aag gcg gat gtg gct act gcg tcg atc aag ggt ttc ttg gag ccg 3701152aag gga ctt ac aac tat gat cca acg oct tgg tat gtg gcc atg tgg 3701152aag gga ctt tac aac tat gat cca acg oct tgg tat gtg gcc atg tgg 177 hys Ala Aap Val Ala Thr Ala Ser Ile Lys Gly Phe Leu Glu Pro 3701200aag gga ctt tac aac tat gat cca acg oct tgg tat gtg gcc atg tgg 198 Gly Leu Tyr Asn Tyr Asp Pro Thr Pro Trp Tyr Val Ala Met Trp 3901200agg gtg gcc aag act tgt cat tat att gag gat gtg gat gga gtc cag 4001248Arg Val Ala Lys Thr Cys His Tyr Ile Glu Asp Val Asp Gly Val Gln 4051296tat tat aag agt ttg gag gat gtg cct ttg aag aag gat gcc aag aag 4001296tct gat tag Ser Asp1305<210> SEQ ID NO 110 <211> LENGTH: 43410<211> LENGTH: 43410<400> SEQUENCE: 11010Met Gly Lys Gly Cly Arg Ser Val Thr Arg Ala Gln Thr Ala Glu Lys 1Met Gly Lys Gly Cly Arg Ser Val Thr Asp Gly Arg Trp Val Ser Pro 20Tyr Asn Pro Leu Ala Lys Asp Ala Pro Glu Leu Pro Ser Lys Gly Glu 30Tyr Asn Pro Leu Ala Lys Asp Ala Pro Glu Leu Pro Ser Lys Gly Glu 4011015Ile Lys Ala Val Ile Pro Lys Glu Cys Phe Glu Arg Ser Tyr Leu His					Gly					Thr					Āla		1008	
Ile Giy Ser Thr His Val Ala His His Leu Phe His Giu Met Pro Phe 35516 Giy Ser Thr His Val Ala His His Leu Phe His Giu Met Pro Phe 365tac aag geg gt gg gt act gg gg to gat c aag ggt ttc ttg gag ccg 3751152Tyr Lys Ala Asp Val Ala Thr Ala Ser Ile Lys Gly Phe Leu Glu Pro 375aag gga ctt tac aact at gat cca acg cct tgg tat gtg gcc atg tgg Lys Gly Leu Tyr Asm Tyr Asp Pro Thr Pro Trp Tyr Val Ala Met Trp 390agg gtg gcc aag act tgt cat tat att gag gat gtg gat gga gtt cag Arg Val Ala Lys Thr Cys His Tyr Ile Glu Asp Val Asp Gly Val Gln 410Ado1248Arg Val Ala Lys Thr Cys His Tyr Ile Glu Asp Val Asp Gly Val Gln 410tat tat aag agt ttg gag gat gtg cct ttg aag aag gat gcc aag aag 4001296Tyr Tyr Lys Ser Leu Glu Asp Val Pro Leu Lys Lys Asp Ala Lys Lys 4201305130541001305130512961296129612961296129612961296129612961296129612961296120Scalag at	-	~		Asp	~	-		~~	Ile		-			His		-	1056	
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202530Tyr Asn Pro Leu Ala Lys Asp Ala Pro Glu Leu Pro Ser Lys Gly Glu 354045Ile Lys Ala Val Ile Pro Lys Glu Cys Phe Glu Arg Ser Tyr Leu His	Met				Gly	Arg	Ser	Val	Thr	-	Ala	Gln	Thr	Ala		Lys		
35 40 45 Ile Lys Ala Val Ile Pro Lys Glu Cys Phe Glu Arg Ser Tyr Leu His	Ser	Ala	His		Ile	Gln	Thr	Phe		Asp	Gly	Arg	Trp		Ser	Pro		
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Ser 65	Met	Tyr	Phe	Val	Leu 70	Arg	Asp	Thr	Val	Met 75	Ala	Val	Ala	Суз	Ala 80
Tyr	Ile	Ala	His	Ser 85	Thr	Leu	Ser	Thr	Asp 90	Ile	Pro	Ser	Glu	Leu 95	Leu
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Ser	His	Ala	Lys	His 165	His	Arg	Arg	Thr	Asn 170	Asn	Ile	Met	Asp	Gly 175	Glu
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Glu	Arg	Ser 195	Gly	Gly	Tyr	Ala	Ala 200	Ile	His	Glu	Ala	Ile 205	Gly	Asp	Gly
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Lys	Glu	Leu	Gln	Ala 245	Gly	Glu	Ile	Ile	Asp 250	His	Tyr	Arg	Pro	Trp 255	Ser
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Lys 385	Gly	Leu	Tyr	Asn	Tyr 390	Asp	Pro	Thr	Pro	Trp 395		Val	Ala	Met	Trp 400
Arg	Val	Ala	Lys	Thr 405		His	Tyr	Ile	Glu 410	Asp	Val	Aap	Gly	Val 415	Gln
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							tcg Ser 200									624
	-	-	-		-	-	tac Tyr			-	-		-			672
							gcc Ala									720
							gcg Ala									768
							tac Tyr									816
-		-			-		gtg Val 280			-			-	-		864
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Ile	Asp	Asn 35	Val	Asp	Ala	Arg	Glu 40	Trp	Ile	Gly	Ala	Leu 45	Ser	Leu	Arg
Leu	Pro 50	Ala	Ile	Ala	Thr	Thr 55	Met	Tyr	Leu	Leu	Phe 60	Суз	Leu	Val	Gly
Pro 65	Arg	Leu	Met	Ala	Lys 70	Arg	Glu	Ala	Phe	Asp 75	Pro	Гла	Gly	Phe	Met 80
Leu	Ala	Tyr	Asn	Ala 85	Tyr	Gln	Thr	Ala	Phe 90	Asn	Val	Val	Val	Leu 95	Gly
Met	Phe	Ala	Arg 100	Glu	Ile	Ser	Gly	Leu 105	Gly	Gln	Pro	Val	Trp 110	Gly	Ser
Thr	Met	Pro 115	Trp	Ser	Asp	Arg	Lys 120	Ser	Phe	Lys	Ile	Leu 125	Leu	Gly	Val
Trp	Leu 130	His	Tyr	Asn	Asn	Lys 135	Tyr	Leu	Glu	Leu	Leu 140	Asp	Thr	Val	Phe
Met 145	Val	Ala	Arg	LYa	Lys 150	Thr	Lys	Gln	Leu	Ser 155	Phe	Leu	His	Val	Tyr 160
His	His	Ala	Leu	Leu 165	Ile	Trp	Ala	Trp	Trp 170	Leu	Val	Сүз	His	Leu 175	Met
Ala	Thr	Asn	Asp 180	Суз	Ile	Asp	Ala	Tyr 185	Phe	Gly	Ala	Ala	Cys 190	Asn	Ser
Phe	Ile	His 195	Ile	Val	Met	Tyr	Ser 200	Tyr	Tyr	Leu	Met	Ser 205	Ala	Leu	Gly
Ile	Arg 210	Суз	Pro	Trp	Lys	Arg 215	Tyr	Ile	Thr	Gln	Ala 220	Gln	Met	Leu	Gln
Phe 225	Val	Ile	Val	Phe	Ala 230	His	Ala	Val	Phe	Val 235	Leu	Arg	Gln	Lys	His 240
Суз	Pro	Val	Thr	Leu 245	Pro	Trp	Ala	Gln	Met 250	Phe	Val	Met	Thr	Asn 255	Met
Leu	Val	Leu	Phe 260	Gly	Asn	Phe	Tyr	Leu 265	ГЛЗ	Ala	Tyr	Ser	Asn 270	Lys	Ser
Arg	Gly	Asp 275	Gly	Ala	Ser	Ser	Val 280	ГЛЗ	Pro	Ala	Glu	Thr 285	Thr	Arg	Ala
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418

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					gat Asp											96		
					acc Thr											144		
					agg Arg											192		
-			-	-	tgt Cys 70					-	-	-				240		
	-	-			ttt Phe		-	-			-					288		
					gtt Val											336		
					tgg Trp											384		
					ttt Phe											432		
					cac His 150											480		
					cca Pro											528		
	-		-		gtt Val		-		-				-		-	576		
					cag Gln											624		
			-		cag Gln		-	-	-						-	672		
			Met	Glu	aat Asn 230	Cys	Pro	Tyr	Gln	Tyr	Pro	-	Phe	-	Tyr	720		
					д1λ ааа											768		
					atc Ile											816		
	~~		-	-	aac Asn					-				-		864		
			-		aac Asn		-	-		-				tga		909		
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<400> SEQUENCE: 118

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Met 1	Ala	Phe	Lys	Glu 5	Leu	Thr	Ser	Arg	Ala 10	Val	Leu	Leu	Tyr	Asp 15	Glu	
Trp	Ile	Lys	Asp 20	Ala	Asp	Pro	Arg	Val 25	Glu	Asp	Trp	Pro	Leu 30	Met	Ser	
Ser	Pro	Ile 35	Leu	Gln	Thr	Ile	Ile 40	Ile	Gly	Ala	Tyr	Ile 45	Tyr	Phe	Val	
Thr	Ser 50	Leu	Gly	Pro	Arg	Ile 55	Met	Glu	Asn	Arg	Lys 60	Pro	Phe	Ala	Leu	
Lуз 65	Glu	Ile	Met	Ala	Cys 70	Tyr	Asn	Leu	Phe	Met 75	Val	Leu	Phe	Ser	Val 80	
Tyr	Met	Суз	Tyr	Glu 85	Phe	Leu	Met	Ser	Gly 90	Trp	Ala	Thr	Gly	Tyr 95	Ser	
Phe	Arg	Cys	Asp 100	Ile	Val	Asp	Tyr	Ser 105	Gln	Ser	Pro	Gln	Ala 110	Leu	Arg	
Met	Ala	Trp 115	Thr	СЛа	Trp	Leu	Phe 120	Tyr	Phe	Ser	ГЛа	Phe 125	Ile	Glu	Leu	
Leu	Asp 130	Thr	Val	Phe	Phe	Val 135	Leu	Arg	ГЛа	ГЛа	Asn 140	Ser	Gln	Ile	Thr	
Phe 145	Leu	His	Val	Tyr	His 150	His	Ser	Ile	Met	Pro 155	Trp	Thr	Trp	Trp	Phe 160	
Gly	Val	ГЛа	Phe	Ala 165	Pro	Gly	Gly	Leu	Gly 170	Thr	Phe	His	Ala	Leu 175	Val	
Asn	Сув	Val	Val 180	His	Val	Ile	Met	Tyr 185	Ser	Tyr	Tyr	Gly	Leu 190	Ser	Ala	
Leu	Gly	Pro 195	Ala	Tyr	Gln	ГЛЗ	Tyr 200	Leu	Trp	Trp	ГЛЗ	Lys 205	Tyr	Met	Thr	
Ser	Ile 210	Gln	Leu	Thr	Gln	Phe 215	Leu	Met	Val	Thr	Phe 220	His	Ile	Gly	Gln	
Phe 225	Phe	Phe	Met	Glu	Asn 230	Сүз	Pro	Tyr	Gln	Tyr 235	Pro	Val	Phe	Leu	Tyr 240	
Val	Ile	Trp	Leu	Tyr 245	Gly	Phe	Val	Phe	Leu 250	Ile	Leu	Phe	Leu	Asn 255	Phe	
Trp	Phe	His	Ala 260	Tyr	Ile	ГЛа	Gly	Gln 265	Arg	Leu	Pro	ГЛа	Ala 270	Val	Gln	
Asn	Gly	His 275	Суз	ГЛа	Asn	Asn	Asn 280	Asn	Gln	Glu	Asn	Thr 285	Trp	Суз	Lys	
Asn	Lys 290	Asn	Gln	ГÀа	Asn	Gly 295	Ala	Leu	ГЛа	Ser	Lуа 300	Asn	His			
<213 <213 <213 <220 <223 <223	L> LI 2> T 3> OF 0> FI L> N 2> LO	ENGTI YPE : RGAN EATUI AME / I OCAT	ISM: RE: KEY: ION:	70 Cio CDS (1)	na in (8' TION	70)			onga	3e						
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					gtg Val											96
cct	aca	att	gct	att	gtg	ttg	ctg	tac	ctg	gcg	ttt	gtt	ctg	tat	att	144

Pro	Thr	Ile 35	Ala	Ile	Val	Leu	Leu 40	Tyr	Leu	Ala	Phe	Val 45	Leu	Tyr	Ile		
				atg Met												19	92
				aac Asn		-	-	-	-						-	24	10
				act Thr 85												28	38
				agt Ser												33	36
	-	-		tgg Trp			-		-				-		-	38	34
				act Thr												43	32
				cat His												48	30
-				ggt Gly 165					-				-			52	28
-	~		-	atc Ile	-		-			~ ~			-			57	76
				aag Lys												62	24
				ttt Phe												67	72
				ccc Pro												72	20
	-			ttc Phe 245		-							-		-	76	58
				tca Ser												81	- 6
				aat Asn												86	54
gat Asp	taa															87	70
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Phe	Ala	Asp	Pro 20	Arg	Val	Ala	Lys	Trp 25	Pro	Leu	Ile	Glu	Asn 30	Pro	Leu		

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

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423		420
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<211 <212 <213 <220 <223 <400	L> LH 2> TY 3> OF 0> FH 3> OT 0> SH	EATUI THER EQUEI	H: 60 DNA ISM: RE: INFO NCE:	0 Art: DRMA 130	TION	: Pr:		a cco	23336	ggat	ccg	gatci	tgc 1	zgget	catgaa	60
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				gcc Ala												96
~ ~	-		~ ~	gcg Ala			000	~		000					-	144
		-		gtc Val	-		-				-					192
				tgg Trp												240
				gtg Val 85												288
				cca Pro												336
				ttc Phe												384
				ctc Leu												432
				atg Met												480
				agc Ser 165												528
-		-		tcg Ser				-	-		-					576
				tgg Trp												624
				gtc Val												672

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											-	con	cin.	uea			
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							act Thr										
	-		-				gct Ala				-				-	768	
				aag Lys		tag										789	
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His	Trp	Asp	Pro 20	Ala	Thr	Thr	Pro	Leu 25	Ala	Ser	Ile	Val	Ser 30	Pro	Сүз		
Val	Ala	Ser 35	Val	Ala	Tyr	Leu	Gly 40	Ala	Ile	Gly	Leu	Leu 45	Lys	Arg	Arg		
Thr	Gly 50	Pro	Glu	Val	Arg	Ser 55	ГÀа	Pro	Phe	Glu	Leu 60	Leu	His	Asn	Gly		
65			-	-	70		Val			75	-			-	80		
				85			Asp		90					95			
-			100				Gln	105	_	_	-		110	-	-		
		115					Ala 120					125					
	130				-	135	Lys				140				-		
145					150		Ile			155					160		
				165			Cys		170					175			
			180				Thr	185					190				
		195					Thr 200 Phe					205					
	210					215	Phe Thr				220						
225	-				230	-	Ala		_	235			-		240		
				245		FIIe	AIA	Бец	250	FIIe	AIG	AIG	Ser	255	Ser		
гда	Pro	ser	Arg 260	Lys	GIU												
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<213		GAN:	ISM:	Eug	lena	gra	cili	3									

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concinaca

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					tcc Ser 70											240
					cag Gln											288
					cca Pro											336
					tac Tyr											384
					cgc Arg											432
					ctg Leu 150											480
					tgg Trp											528
					tat Tyr											576
tgg Trp	tgg Trp	aag Lys 195	aag Lys	tgg Trp	atg Met	acc Thr	acc Thr 200	atg Met	cag Gln	atc Ile	atc Ile	cag Gln 205	ttc Phe	atc Ile	acg Thr	624
					acg Thr											672
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<212> TYPE: PRT <213> ORGANISM: Euglena gracilis

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Val Ala Ser Val 3 35	Ala Tyr Le	u Gly Ala 40	Ile Gly Le	eu Leu Lys 45	Arg Arg
Thr Gly Pro Glu 50	Val Arg Se: 55	Lys Pro	Phe Glu Le 60		Asn Gly
Leu Leu Val Gly ' 65	Trp Ser Lev 70	ı Val Val	Leu Leu G 75	ly Thr Leu	Tyr Gly 80
Ala Tyr Gln Arg	Val Gln Gl 85		Arg Gly Va 90	al Gln Ala	Leu Leu 95
Cys Thr Gln Arg 1 100	Pro Pro Se	Gln Ile 105	Trp Asp G	ly Pro Val 110	Gly Tyr
Phe Thr Tyr Leu 1 115	Phe Tyr Le	1 Ala Lys 120	Tyr Trp G	lu Leu Val 125	Asp Thr
Val Ile Leu Ala 1 130	Leu Arg Gli 13	-		ro Leu His 40	Val Tyr
His His Ala Val 145	Met Leu Pho 150	e Ile Val	Trp Ser Ti 155	rp Phe Ala	His Pro 160
Trp Leu Glu Gly :	Ser Trp Trj 165	-	Leu Val As 170	sn Ser Phe	Ile His 175
Thr Val Met Tyr : 180	Ser Tyr Ty:	Thr Leu 185	Thr Val Vá	al Gly Ile 190	Asn Pro
Trp Trp Lys Lys ' 195	Trp Met Th	Thr Met 200	Gln Ile II	le Gln Phe 205	Ile Thr
Gly Cys Val Tyr 210	Val Thr Al. 21			yr Tyr Ala 20	Gly Ala
Gly Cys Thr Ser 2 225	Asn Val Ty: 230	Thr Ala	Trp Phe Se 235	er Met Gly	Val Asn 240
Leu Ser Phe Leu '	Trp Leu Pho 245		Phe Phe Ai 250	rg Arg Ser	Tyr Ser 255
Lys Pro Ser Arg 1 260	Lys Glu				
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gtt ttc ttt gtc 1 Val Phe Phe Val 1 35					
ctc ctc cga tac a	acc gtc ga	tca ctc	ccc aca ct	tc ggt ccc	cgc att 192

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													CIII				
Leu	Leu 50	Arg	Tyr	Thr	Val	Asp 55	Ser	Leu	Pro	Thr	Leu 60	Gly	Pro	Arg	Ile		
					gcc Ala 70	-		-								240	
		-	-	-	ggt Gly	-								-	-	288	
					ttc Phe		~		<u> </u>							336	
					ttc Phe											384	
				-	gac Asp											432	
					ctc Leu 150											480	
					tta Leu											528	
					acg Thr											576	
					tcg Ser											624	
					ttt Phe											672	
-					tat Tyr 230			-		-	-					720	
•					gtg Val			•				<u> </u>				768	
					aac Asn											816	
					ata Ile											864	
					tcc Ser	-			-	taa						897	
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Val	Phe	Phe 35	Val	Phe	Val	Val	Val 40	Ser	Leu	Tyr	Leu	Ser 45	Ala	Thr	Phe		

Leu Leu Arg Tyr Thr Val Asp Ser Leu Pro Thr Leu Gly Pro Arg Ile Leu Lys Pro Ile Thr Ala Val His Ser Leu Ile Leu Phe Leu Leu Ser Leu Thr Met Ala Val Gly Cys Thr Leu Ser Leu Ile Ser Ser Asp Pro Lys Ala Arg Leu Phe Asp Ala Val Cys Phe Pro Leu Asp Val Lys Pro Lys Gly Pro Leu Phe Phe Trp Ala Gln Val Phe Tyr Leu Ser Lys Ile Leu Glu Phe Val Asp Thr Leu Leu Ile Ile Leu Asn Lys Ser Ile Gln Arg Leu Ser Phe Leu His Val Tyr His His Ala Thr Val Val Ile Leu Cys Tyr Leu Trp Leu Arg Thr Arg Gln Ser Met Phe Pro Val Gly 165 170 Leu Val Leu Asn Ser Thr Val His Val Ile Met Tyr Gly Tyr Tyr Phe 180 185 Leu Cys Ala Ile Gly Ser Arg Pro Lys Trp Lys Lys Leu Val Thr Asn Phe Gln Met Val Gln Phe Ala Phe Gly Met Gly Leu Gly Ala Ala Trp Met Leu Pro Glu His Tyr Phe Gly Ser Gly Cys Ala Gly Ile Trp Thr Val Tyr Phe Asn Gly Val Phe Thr Ala Ser Leu Leu Ala Leu Phe Tyr Asn Phe His Ser Lys Asn Tyr Glu Lys Thr Thr Thr Ser Pro Leu Tyr Lys Ile Glu Ser Phe Ile Phe Ile His Gly Glu Arg Trp Ala Asn Lys Ala Ile Thr Leu Phe Ser Lys Lys Asn Asp <210> SEQ ID NO 137 <211> LENGTH: 837 <212> TYPE: DNA <213> ORGANISM: Arabidopsis thaliana <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (1)..(837) <223> OTHER INFORMATION: Delta-5 elongase <400> SEQUENCE: 137 atg gca tca att tac tcc tct tta acc tac tgg ctc gtt aac cac ccc Met Ala Ser Ile Tyr Ser Ser Leu Thr Tyr Trp Leu Val Asn His Pro tac atc tcc aat ttt act tgg atc gaa ggt gaa acc cta ggc tcc acc Tyr Ile Ser Asn Phe Thr Trp Ile Glu Gly Glu Thr Leu Gly Ser Thr gtc ttt ttc gta tcc gtc gta gtc tcc gtt tac ctc tcc gcc acg ttc Val Phe Phe Val Ser Val Val Val Ser Val Tyr Leu Ser Ala Thr Phe ctc ctc cga tcc gcc atc gat tca ctc cca tca ctc agt cca cgt atc Leu Leu Arg Ser Ala Ile Asp Ser Leu Pro Ser Leu Ser Pro Arg Ile ctc aaa ccg atc aca gcc gtc cac agc cta atc ctc tgt ctc ctc tcc Leu Lys Pro Ile Thr Ala Val His Ser Leu Ile Leu Cys Leu Leu Ser

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											-	con	tin	ued		
65					70					75					80	
	<u> </u>			<u> </u>			act Thr									
		-	-	-		-	ttc Phe					-			-	
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							tgg Trp									
							tcg Ser									
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Val	Phe	Phe 35	Val	Ser	Val	Val	Val 40	Ser	Val	Tyr	Leu	Ser 45	Ala	Thr	Phe	
Leu	Leu 50	Arg	Ser	Ala	Ile	Asp 55	Ser	Leu	Pro	Ser	Leu 60	Ser	Pro	Arg	Ile	
Leu 65	ГЛа	Pro	Ile	Thr	Ala 70	Val	His	Ser	Leu	Ile 75	Leu	Суа	Leu	Leu	Ser 80	
Leu	Val	Met	Ala	Val 85	Gly	Сүз	Thr	Leu	Ser 90	Ile	Thr	Ser	Ser	His 95	Ala	L. C.

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Ser Ser	Asp	Pro 100	Met	Ala	Arg	Phe	Leu 105	His	Ala	Ile	Сүз	Phe 110	Pro	Val
Asp Val	Lys 115	Pro	Asn	Gly	Pro	Leu 120	Phe	Phe	Trp	Ala	Gln 125	Val	Phe	Tyr
Leu Ser 130	Lys	Ile	Leu	Glu	Phe 135	Gly	Asp	Thr	Ile	Leu 140	Ile	Ile	Leu	Gly
Lys Ser 145	Ile	Gln	Arg	Leu 150	Ser	Phe	Leu	His	Val 155	Tyr	His	His	Ala	Thr 160
Val Val	Val	Met	Cys 165	Tyr	Leu	Trp	Leu	Arg 170	Thr	Arg	Gln	Ser	Met 175	Phe
Pro Ile	Ala	Leu 180	Val	Thr	Asn	Ser	Thr 185	Val	His	Val	Ile	Met 190	Tyr	Gly
Tyr Tyr	Phe 195	Leu	Суз	Ala	Val	Gly 200	Ser	Arg	Pro	Гла	Trp 205	Lys	Arg	Leu
Val Thr 210	Asp	Суз	Gln	Ile	Val 215	Gln	Phe	Val	Phe	Ser 220	Phe	Gly	Leu	Ser
Gly Trp 225	Met	Leu	Arg	Glu 230	His	Leu	Phe	Gly	Ser 235	Gly	Суа	Thr	Gly	Ile 240
Trp Gly	Trp	Суз	Phe 245	Asn	Ala	Ala	Phe	Asn 250	Ala	Ser	Leu	Leu	Ala 255	Leu
Phe Ser	Asn	Phe 260	His	Ser	Lys	Asn	Tyr 265	Val	Lys	Lys	Pro	Thr 270	Arg	Glu
Asp Gly	Lys 275	Lys	Ser	Asb										
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<210> SH <211> LH <212> TY <213> OH <220> FH <223> OT <220> FH	ENGTH YPE : RGANI EATUR THER	I: 8 PRT SM: E: INFC	Arti			_		seque	ence					
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<223> 01	THER ne, p EATUR AME/K	INFO refe E: EY:	ORMAT erabl MISC	TION Ly is C_FEA	: Xaa 3 Ala ATURE	a or		. Су:	5, Le	eu, 1	Met,	Ala,	, Ile	e, Val or

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tattgaccgt gcacttgtgt aaaacagaga tatttcaaga gt atg a Met 1 1	atg gta cct 114 Met Val Pro
tae and tet man men tot oth ath oth ath one are tot	r and that 160
cca agt tat gac gag tat atc gtc atg gtc aac gac ctt Ser Ser Tyr Asp Glu Tyr Ile Val Met Val Asn Asp Leu	

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5					10					15					20		
att ci Ile Le	-	-		-	-		-			-						210	
tgg ga Trp G				•			•	•				•	•			258	
ctc ci Leu Le	eu															306	
gca at Ala II 70	le															354	
atg ci Met Le 85																402	
aac ge Asn G	-						-		-		-		-	-	-	450	
cct at Pro I																498	
ttt to Phe T:	rp	-					-			-	-		-			546	
tcc ti Ser Pl 1!		-		-												594	
ttg aa Leu As 165																642	
ttg aa Leu As																690	
atg ca Met H:																738	
aaa to Lys Se	er															786	
acg ca Thr G 23																834	
cgg g Arg Va 245						Leu										882	
ttc go Phe Al																930	
aca go Thr Al		taa	gcga	aati	ttg 🤉	ggtc	tacgi	tt aa	aaaca	aatta	a cgi	ttaca	aaaa			979	
aaaaaa	iaaa	iaa a	aaaa													993	
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His	Thr	Glu 35	Gly	Trp	Glu	Phe	Thr 40	Asp	Phe	Ser	Ala	Ala 45	Phe	Ser	Ile
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Met 65	Gly	Val	Pro	Ala	Ile 70	Asp	Pro	Tyr	Pro	Leu 75	ГЛа	Phe	Val	Tyr	Asn 80
Val	Ser	Gln	Ile	Met 85	Leu	Суз	Ala	Tyr	Met 90	Thr	Ile	Glu	Ala	Ser 95	Leu
Leu	Ala	Tyr	Arg 100	Asn	Gly	Tyr	Thr	Phe 105	Trp	Pro	Суз	Asn	Asp 110	Trp	Asp
Phe	Glu	Lys 115	Pro	Pro	Ile	Ala	Lys 120	Leu	Leu	Trp	Leu	Phe 125	Tyr	Val	Ser
LÀa	Ile 130	Trp	Aab	Phe	Trp	Asp 135	Thr	Ile	Phe	Ile	Val 140	Leu	Gly	Lys	Lys
Trp 145	Arg	Gln	Leu	Ser	Phe 150	Leu	His	Val	Tyr	His 155	His	Thr	Thr	Ile	Phe 160
Leu	Phe	Tyr	Trp	Leu 165	Asn	Ala	His	Val	Asn 170	Phe	Asp	Gly	Asp	Ile 175	Phe
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Tyr	Phe	Ile 195	Cys	Met	His	Thr	Lys 200	Val	Pro	Glu	Thr	Gly 205	Lys	Ser	Leu
Pro	Ile 210	Trp	Trp	Lys	Ser	Ser 215	Leu	Thr	Ser	Met	Gln 220	Leu	Val	Gln	Phe
Ile 225	Thr	Met	Met	Thr	Gln 230	Ala	Ile	Met	Ile	Leu 235	Tyr	Гла	Gly	Суз	Ala 240
Ala	Pro	His	Ser	Arg 245	Val	Val	Thr	Ser	Tyr 250	Leu	Val	Tyr	Ile	Leu 255	Ser
Leu	Phe	Ile	Leu 260	Phe	Ala	Gln	Phe	Phe 265	Val	Ser	Ser	Tyr	Leu 270	Lys	Pro
ГЛа	Lys	Lys 275	Lys	Thr	Ala										
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<400)> SI	EQUEI	ICE :	185											
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tttctcttct actggttgaa tgcacatgta aactttgatg gtgatatttt cctcaccatc	180
gtettgaaeg gttteateea eacegteatg taeaegtaet aetteatttg eatgeaeaee	240
aaggtcccag agaccggcaa atccttgccc atttggtgga aatctagttt gacaagcatg	300
cagetggtge agtteateae gatgatgaeg eaggetatea tgatettgta eaagggetgt	360
gctgctcccc atagccgggt ggtgacatcg tacttggttt acattttgtc gctctttatt	420
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<223> OTHER INFORMATION: Primer

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jaagacaget taataggegg eege	24
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atgggagttc actgactttt ctgctgcttt tagcattgcc gtcgcgtacc tcctgtttgt	180
sttigtigga teteteatta tgagtatggg agteeeegea attgaeeett ateegeteaa	240
yttgtctac aatgtttcac agattatgct ttgtgcttac atgaccattg aagccagtct	300
ctagcttat cgtaacggct acacattctg gccttgcaac gattgggact ttgaaaagcc	360
jectateget aageteetet ggetetttta egttteeaaa atttgggatt tttgggacae	420
atetttatt gtteteggga agaagtggeg teaaetttee tteetgeaeg tetaeeatea	480
accaccatc tttctcttct actggttgaa tgcacatgta aactttgatg gtgatatttt	540
ectcaccate gtettgaaeg gttteateea eacegteatg taeaegtaet aetteatttg	600
atgcacacc aaggtcccag agaccggcaa atccttgccc atttggtgga aatctagttt	660
jacaagcatg cagctggtgc agttcatcac gatgatgacg caggctatca tgatcttgta	720
aagggetgt getgeteeee atageegggt ggtgaeateg taettggttt aeattttgte	780
getetttatt ttgttegeee agttetttgt eageteatae eteaageega agaagaagaa	840
jacagettaa tagaetagt	859
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atc cgc gag cac gcg acg ccc gcg acc gcg tgg atc gtg att cac cac Cle Arg Glu His Ala Thr Pro Ala Thr Ala Trp Ile Val Ile His His 20 25 30	96
aag gtc tac gac atc tcc aag tgg gac tcg cac ccg ggt ggc tcc gtg ys Val Tyr Asp Ile Ser Lys Trp Asp Ser His Pro Gly Gly Ser Val 35 40 45	144
atg ctc acg cag gcc ggc gag gac gcc acg gac gcc ttc gcg gtc ttc Met Leu Thr Gln Ala Gly Glu Asp Ala Thr Asp Ala Phe Ala Val Phe 50 55 60	192
vac ccg tcc tcg gcg ctc aag ctg ctc gag cag ttc tac gtc ggc gac His Pro Ser Ser Ala Leu Lys Leu Leu Glu Gln Phe Tyr Val Gly Asp 55 70 75 80	240
gtg gac gaa acc tcc aag gcc gag atc gag ggg gag ccg gcg agc gac Val Asp Glu Thr Ser Lys Ala Glu Ile Glu Gly Glu Pro Ala Ser Asp	288

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_												con	tin	ued			
				85					90					95			
	gag Glu															336	
	cgt Arg			•		•				000				•	•	384	
	ctc Leu 130					-			-	-						432	
	tcg Ser	-			-					-		-	-		-	480	
	gcc Ala															528	
	cac His	-		-			-		-			-	-			576	
	ctt Leu			-					-		-			-	-	624	
	tgg Trp 210															672	
	agc Ser															720	
-	ccg Pro	-	-				_		-		-	-				768	
	gcg Ala															816	
	ctg Leu															864	
	gtg Val 290					-					-	-	-			912	
	gga Gly															960	
	atc Ile															1008	
	ctc Leu															1056	
-	att Ile					-	-				-	-		-	-	1104	
	ttc Phe 370															1152	
-	ttc Phe	-	-						-			-		-		1200	
cac	ctg	ttc	ccg	ctc	gtg	ccg	cgc	cac	aac	ttg	cca	aag	gtc	aac	gtg	1248	

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His	Leu	Phe	Pro	Leu 405	Val	Pro	Arg	His	Asn 410	Leu	Pro	Lys	Val	Asn 415	Val			
				cta Leu												1296		
				ggc Gly												1344		
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Ile	Arg	Glu	His 20	Ala	Thr	Pro	Ala	Thr 25	Ala	Trp	Ile	Val	Ile 30	His	His			
Lys	Val	Tyr 35	Asp	Ile	Ser	ГЛа	Trp 40	Asp	Ser	His	Pro	Gly 45	Gly	Ser	Val			
Met	Leu 50	Thr	Gln	Ala	Gly	Glu 55	Asp	Ala	Thr	Asp	Ala 60	Phe	Ala	Val	Phe			
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Glu	Glu	Arg	Ala 100	Arg	Arg	Glu	Arg	Ile 105	Asn	Glu	Phe	Ile	Ala 110	Ser	Tyr			
Arg	Arg	Leu 115	Arg	Val	Lys	Val	Lys 120	Gly	Met	Gly	Leu	Tyr 125	Asp	Ala	Ser			
Ala	Leu 130	Tyr	Tyr	Ala	Trp	Lys 135	Leu	Val	Ser	Thr	Phe 140	Gly	Ile	Ala	Val			
Leu 145	Ser	Met	Ala	Ile	Cys 150	Phe	Phe	Phe	Asn	Ser 155	Phe	Ala	Met	Tyr	Met 160			
Val	Ala	Gly		Ile 165							Gln		-	_	Leu			
Ala	His	Aap	Phe 180	Leu	His	Asn	Gln	Val 185	Суз	Glu	Asn	Arg	Thr 190	Leu	Gly			
Asn	Leu	Ile 195	Gly	Суз	Leu	Val	Gly 200	Asn	Ala	Trp	Gln	Gly 205	Phe	Ser	Met			
Gln	Trp 210	Trp	Lys	Asn	Lys	His 215	Asn	Leu	His	His	Ala 220	Val	Pro	Asn	Leu			
His 225	Ser	Ala	Lys	Asp	Glu 230	Gly	Phe	Ile	Gly	Asp 235	Pro	Asp	Ile	Asp	Thr 240			
Met	Pro	Leu	Leu	Ala 245	Trp	Ser	Гла	Glu	Met 250	Ala	Arg	ГЛа	Ala	Phe 255	Glu			
Ser	Ala	His	Gly 260	Pro	Phe	Phe	Ile	Arg 265	Asn	Gln	Ala	Phe	Leu 270	Tyr	Phe			
Pro	Leu	Leu 275	Leu	Leu	Ala	Arg	Leu 280	Ser	Trp	Leu	Ala	Gln 285	Ser	Phe	Phe			
Tyr	Val 290		Thr	Glu	Phe			Gly	Ile	Phe	-		Val	Glu	Phe			
	290					295					300							

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Asp 305																
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Phe	Leu	Met	Gly 340	Gln	Ala	Ser	Суз	Gly 345	Leu	Leu	Leu	Ala	Leu 350	Val	Phe	
Ser	Ile	Gly 355	His	Asn	Gly	Met	Ser 360	Val	Tyr	Glu	Arg	Glu 365	Thr	Lys	Pro	
Asp	Phe 370	Trp	Gln	Leu	Gln	Val 375	Thr	Thr	Thr	Arg	Asn 380	Ile	Arg	Ala	Ser	
Val 385	Phe	Met	Asp	Trp	Phe 390	Thr	Gly	Gly	Leu	Asn 395	Tyr	Gln	Ile	Asp	His 400	
His	Leu	Phe	Pro	Leu 405	Val	Pro	Arg	His	Asn 410	Leu	Pro	Lys	Val	Asn 415	Val	
Leu	Ile	Lys	Ser 420	Leu	СЛа	LÀa	Glu	Phe 425	Asp	Ile	Pro	Phe	His 430	Glu	Thr	
Gly	Phe	Trp 435	Glu	Gly	Ile	Tyr	Glu 440	Val	Val	Asp	His	Leu 445	Ala	Asb	Ile	
Ser	Lys 450	Glu	Phe	Ile	Thr	Glu 455	Phe	Pro	Ala	Met						
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	0> SI	EQUEI						12 u	Ball	IIAS	2					
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	cac His															480
	ttg Leu															528
	ggc Gly															576
	tac Tyr															624
	cat His 210															672
	atc Ile															720
	cga Arg															768
	ggc Gly															816
	caa Gln															864
-	tgg Trp 290		-		-					-	-	-				912
	aac Asn															960
	ttc Phe															1008
	aaa Lys	-		-		-			-		-			-		1056
	aag Lys															1104
-	gag Glu 370				-		-				-		-		taa	1152
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	3 > OF				∋ndu∶	la oi	Efic:	inal:	is							
)> SH															
1	Gly		-	5	-			-	10			_	-	15	-	
Thr	Glu	Pro	Glu 20	Pro	Ile	Gln	Arg	Val 25	Pro	His	Glu	ГÀа	Pro 30	Pro	Phe	
Thr	Val	Gly 35	Asp	Ile	Lys	Lys	Ala 40	Ile	Pro	Pro	His	Суз 45	Phe	Asn	Arg	
Ser	Val 50	Ile	Arg	Ser	Phe	Ser 55	Tyr	Val	Phe	Tyr	Asp 60	Leu	Thr	Ile	Ala	

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Ser 65	Ile	Leu	Tyr	Tyr	Ile 70	Ala	Asn	Asn	Tyr	Ile 75	Ser	Thr	Leu	Pro	Ser 80
Pro	Leu	Ala	Tyr	Val 85	Ala	Trp	Pro	Val	Tyr 90	Trp	Ala	Val	Gln	Gly 95	Сүз
Val	Leu	Thr	Gly 100	Val	Trp	Val	Ile	Ala 105	His	Glu	Суз	Gly	His 110	His	Ala
Phe	Ser	Asp 115	His	Gln	Trp	Leu	Asp 120	Asp	Thr	Val	Gly	Leu 125	Val	Leu	His
Ser	Phe 130	Leu	Leu	Val	Pro	Tyr 135	Phe	Ser	Trp	Lys	Tyr 140	Ser	His	Arg	Arg
His 145	His	Ser	Asn	Thr	Gly 150	Ser	Ile	Glu	His	Asp 155	Glu	Val	Phe	Val	Pro 160
Lys	Leu	Lys	Ser	Gly 165	Val	Arg	Ser	Thr	Ala 170	Arg	Tyr	Leu	Asn	Asn 175	Pro
Pro	Gly	Arg	Ile 180	Leu	Thr	Leu	Leu	Val 185	Thr	Leu	Thr	Leu	Gly 190	Trp	Pro
Leu	Tyr	Leu 195	Thr	Phe	Asn	Val	Ser 200	Gly	Arg	Tyr	Tyr	Asp 205	Arg	Phe	Ala
Cys	His 210	Phe	Asp	Pro	Asn	Ser 215	Pro	Ile	Tyr	Ser	Lys 220	Arg	Glu	Arg	Ala
Gln 225	Ile	Phe	Ile	Ser	Asp 230	Ala	Gly	Ile	Leu	Ala 235	Val	Val	Phe	Val	Leu 240
Phe	Arg	Leu	Ala	Met 245	Thr	Lys	Gly	Leu	Thr 250	Trp	Val	Leu	Thr	Met 255	Tyr
Gly	Gly	Pro	Leu 260	Leu	Val	Val	Asn	Gly 265	Phe	Leu	Val	Leu	Ile 270	Thr	Phe
Leu	Gln	His 275	Thr	His	Pro	Ser	Leu 280	Pro	His	Tyr	Asp	Ser 285	Thr	Glu	Trp
Asp	Trp 290	Leu	Arg	Gly	Ala	Leu 295	Thr	Thr	Ile	Asp	Arg 300	Asp	Tyr	Gly	Ile
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Phe	Lys	Ala 355	Met	Tyr	Arg	Glu	Thr 360	Lys	Glu	Cys	Ile	Tyr 365	Val	Asp	Гла
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48

475

476

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	•				gct Ala				•					144	
Leu		-		-	acc Thr	-		-		-				192	
	-	-	-	-	aag Lys 70	 	-	-		-			-	240	
					tac Tyr									288	
					atc Ile									336	
	-			-	gat Asp	 -		_		-	-			384	
Trp					aat Asn									432	
-		-	00		aag Lys 150	-	-			-		0.0		480	
		-	-	-	att Ile	 -			-	-			-	528	
					atc Ile									576	
					atg Met									624	
Ile .					aag Lys									672	
					gct Ala 230	-	-			-		-		720	
					cct Pro									768	
					aac Asn									816	
00		<u> </u>	00	<u> </u>	tct Ser	<u> </u>	-	<u> </u>	0.0			-	0	864	
					acc Thr					tga				903	

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Leu	Pro 50	Ala	Ile	Ala	Thr	Thr 55	Met	Tyr	Leu	Leu	Phe 60	Суз	Leu	Val	Gly		
Pro 65	Arg	Leu	Met	Ala	Lys 70	Arg	Glu	Ala	Phe	Asp 75	Pro	Lys	Gly	Phe	Met 80		
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Met	Phe	Ala	Arg 100		Ile	Ser	Gly	Leu 105	Gly	Gln	Pro	Val	Trp 110	Gly	Ser		
Thr	Met	Pro 115	Trp	Ser	Asp	Arg	Lys 120	Ser	Phe	Lys	Ile	Leu 125	Leu	Gly	Val		
Trp	Leu 130		Tyr	Asn	Asn	Lys 135	Tyr	Leu	Glu	Leu	Leu 140	Asp	Thr	Val	Phe		
Met 145	Val	Ala	Arg	Lys	Lys 150		Lys	Gln	Leu	Ser 155	Phe	Leu	His	Val	Tyr 160		
His	His	Ala	Leu	Leu 165	Ile	Trp	Ala	Trp	Trp 170	Leu	Val	Сүв	His	Leu 175	Met		
Ala	Thr	Asn	Asp 180	Сүз	Ile	Aap	Ala	Tyr 185	Phe	Gly	Ala	Ala	Cys 190	Asn	Ser		
Phe	Ile	His 195	Ile	Val	Met	Tyr	Ser 200	Tyr	Tyr	Leu	Met	Ser 205	Ala	Leu	Gly		
Ile	Arg 210		Pro	Trp	Гла	Arg 215	Tyr	Ile	Thr	Gln	Ala 220	Gln	Met	Leu	Gln		
Phe 225	Val	Ile	Val	Phe	Ala 230	His	Ala	Val	Phe	Val 235	Leu	Arg	Gln	Lys	His 240		
Суз	Pro	Val	Thr	Leu 245	Pro	Trp	Ala	Gln	Met 250	Phe	Val	Met	Thr	Asn 255	Met		
Leu	Val	Leu	Phe 260	Gly	Asn	Phe	Tyr	Leu 265	Lys	Ala	Tyr	Ser	Asn 270	Lys	Ser		
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	gat Asp		-			-					-	-	-			96	
-	tgg Trp	-				-					-		-		-	144	

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	gag Glu 50															192	
	gtg Val															240	
	aga Arg															288	
	ttg Leu															336	
	tac Tyr															384	
	acc Thr 130		-	-										-		432	
	gag Glu			-				-			-					480	
	gtg Val			-												528	
	tgg Trp			-	-	-	-				-	-				576	
-	gct Ala					-			-	-						624	
	tct Ser 210															672	
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	gtg Val															768	
	ctc Leu															816	
	gga Gly															864	
	aag Lys 290			tga												879	
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)> SH					P	7	P ¹	T -		7	D ¹		m'	T		
1	Ser			5					10					15			
Trp	Asp	Tyr	Ala 20	⊥le	Ser	гла	Va⊥	Val 25	Pne	Thr	Суз	Ala	Asp 30	Ser	Phe		

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Gln	Trp	Asp 35	Ile	Gly	Pro	Val	Ser 40	Ser	Ser	Thr	Ala	His 45	Leu	Pro	Ala				
Ile	Glu 50	Ser	Pro	Thr	Pro	Leu 55	Val	Thr	Ser	Leu	Leu 60	Phe	Tyr	Leu	Val				
Thr 65	Val	Phe	Leu	Trp	Tyr 70	Gly	Arg	Leu	Thr	Arg 75	Ser	Ser	Asb	Lys	Lys 80				
Ile	Arg	Glu	Pro	Thr 85	Trp	Leu	Arg	-	Phe 90	Ile	Ile	Суз	His	Asn 95	Ala				
Phe	Leu	Ile	Val 100	Leu	Ser	Leu	Tyr	Met 105	Суз	Leu	Gly	Суз	Val 110	Ala	Gln				
Ala	Tyr	Gln 115		Gly	Tyr	Thr	Leu 120		Gly	Asn	Glu	Phe 125		Ala	Thr				
Glu	Thr 130		Leu	Ala	Leu	Tyr 135		Tyr	Ile	Phe	Tyr 140		Ser	Lys	Ile				
Tyr 145		Phe	Val	Asp	Thr 150		Ile	Met	Leu	Leu 155		Asn	Asn	Leu	Arg 160				
	Val	Ser	Phe	Leu 165	His	Ile	Tyr	His	His 170		Thr	Ile	Ser	Phe 175					
Trp	Trp	Ile			Arg	Arg	Ala			Gly	Asp	Ala	-		Ser				
Ala	Ala		180 Asn	Ser	Trp	Val		185 Val	Суз	Met	Tyr		190 Tyr	Tyr	Leu				
Leu		195 Thr	Leu	Ile	Gly	-	200 Glu	Asp	Pro	Lys	-	205 Ser	Asn	Tyr	Leu				
_	210 Trp	Gly	Arg	His	Leu	215 Thr	Gln	Met	Gln		220 Leu	Gln	Phe	Phe					
225 Asn	Val	Leu	Gln		230 Leu	Tyr	Суз	Ala	Ser	235 Phe	Ser	Thr	Tyr	Pro	240 Lys				
Phe	Leu	Ser	Lys	245 Ile	Leu	Leu	Val	Tyr	250 Met	Met	Ser	Leu	Leu	255 Gly	Leu				
Phe	Gly	His	260 Phe	Tyr	Tyr	Ser	Lys	265 His	Ile	Ala	Ala	Ala	270 Lys	Leu	Gln				
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1	290																		
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		EQUEN						.									- 0		
yyat	.cett	.aa t	.caaç	Jaca	cg co	Jaaa			gtt Val							ţ	52		
					gag Glu 15		-		-			-	-	-	-	10	00		
					ttg Leu											14	48		
					aga Arg											19	96		

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													CIII			
		acc Thr 60														244
		aac Asn														292
	-	tct Ser	-	-	-		-	-	-	-	-	-			-	340
		aag Lys														388
		tgg Trp														436
		cat His 140														484
		acc Thr														532
		gct Ala														580
		cat His														628
	-	ttc Phe		-				-	-				-	-		676
		atg Met 220														724
		gat Asp														772
		gat Asp		-						-				-	-	820
		tgg Trp														868
		ttc Phe														916
		ctt Leu 300														964
	-	gct Ala	-					-	-							1012
-	-	act Thr			-			-		-		-				1060
		cac His			-	-	-	-		-	-			-		1108
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							caa Gln									1252	
							aag Lys									1300	
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							gga Gly								tga	1396	
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Ala	Phe	Asp	Gly 20	Glu	Arg	Glu	Arg	Ala 25	Glu	Ala	Asn	Val	Lуа 30	Leu	Ser		
Ala	Glu	Lys 35	Met	Glu	Pro	Ala	Ala 40	Leu	Ala	Lys	Thr	Phe 45	Ala	Arg	Arg		
Tyr	Val 50	Val	Ile	Glu	Gly	Val 55	Glu	Tyr	Asp	Val	Thr 60	Asp	Phe	Lys	His		
Pro 65	Gly	Gly	Thr	Val	Ile 70	Phe	Tyr	Ala	Leu	Ser 75	Asn	Thr	Gly	Ala	Asp 80		
Ala	Thr	Glu	Ala	Phe 85	Lys	Glu	Phe	His	His 90	Arg	Ser	Arg	Lys	Ala 95	Arg		
Lya	Ala	Leu	Ala 100	Ala	Leu	Pro	Ser	Arg 105	Pro	Ala	ГЛа	Thr	Ala 110	Lys	Val		
Asp	Asp	Ala 115	Glu	Met	Leu	Gln	Asp 120	Phe	Ala	Lys	Trp	Arg 125	Lys	Glu	Leu		
Glu	Arg 130	Asp	Gly	Phe	Phe	Lys 135	Pro	Ser	Pro	Ala	His 140	Val	Ala	Tyr	Arg		
Phe 145	Ala	Glu	Leu	Ala	Ala 150	Met	Tyr	Ala	Leu	Gly 155	Thr	Tyr	Leu	Met	Tyr 160		
Ala	Arg	Tyr	Val	Val 165	Ser	Ser	Val	Leu	Val 170	Tyr	Ala	СЛа	Phe	Phe 175	Gly		
Ala	Arg	Сув	Gly 180	Trp	Val	Gln	His	Glu 185	Gly	Gly	His	Ser	Ser 190	Leu	Thr		
Gly	Asn	Ile 195	Trp	Trp	Asp	Lys	Arg 200	Ile	Gln	Ala	Phe	Thr 205	Ala	Gly	Phe		
Gly	Leu 210	Ala	Gly	Ser	Gly	Asp 215	Met	Trp	Asn	Ser	Met 220	His	Asn	Гла	His		
His 225	Ala	Thr	Pro	Gln	Lys 230	Val	Arg	His	Asp	Met 235	Asp	Leu	Asp	Thr	Thr 240		
Pro	Ala	Val	Ala	Phe 245	Phe	Asn	Thr	Ala	Val 250	Glu	Asp	Asn	Arg	Pro 255	Arg		

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275 280 285 Ser Lys Ala Leu Lys Gly Gly Lys Tyr Glu Glu Soo Val Trp Met Leu Ala Ala Ala Mis Val Ile Arg Thr Thr The Lus Ala Thi Soo Val Thr Met Leu Ala Ala Met Gln Ser 310 Th Thr The Lus Ala Thr Ser 320 Thr Ala Met Gln Ser Thr Ser 345 Ser Gly Cys Tyr Leu Phe Ala His Ser Thr Ser Gly Val Pro Ala Asp Glu His Leu Ser Thr Ser 355 Val Val Pro Ala Asp Glu Ser Thr Val Arg Tyr Ala Asp 370 Thr Ser Se	
290 295 300 Ala Ala His Val Ile Arg Th Th Ile Ala Val Th So Th Ala His Val Ile Arg Th Th Th So So Th Ala His Val Ile Arg Th Ile Arg Ala Val Fhe So Th Ala Mis Gu So So So So So So So Th Ala Mis So So So So So So So Gu Val So So So So Th So Fue So So So Val So So So So So So So So So So Val So So So So So So So So So So So So So So So So	
305 310 315 320 Thr Ala Met Gln Ser 320 Met Ser 320 Gly Yar Leu Pho Ala Thr Ser Yal Ser Gly Yar Leu Pho Ala His Ser Thr Ser Ser Val Val Ser Ala Ne Ser Thr Ser Ser Val Val Pro Ala Ala Ser Thr Ser Ala Ser Val Val Ser Ala Ser Thr Ser Thr Ser Ser Val Val Ser Ala Ser Thr Ser Thr Ser Ser Sab Ser Ser Thr Val Arg Thr Ser Ser Ser Sab Ser	
325 330 335 Gly Cys Tyr Leu Phe Ala His Ala Sub Ala Sub Ala Sub Ala Ala Sub Ala Ala Sub Ala Ala Sub Ala	
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Gln Phe Arg Gln Pro Glu Val Ser Arg Arg Phe Val Ala Phe Ala Lys 405 410 415	
Lys Trp Asn Leu Asn Tyr Lys Val Met Thr Tyr Ala Gly Ala Trp Lys 420 425 430	
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<213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: His box3 <400> SEQUENCE: 250 Gln Ile Glu His His Leu Phe Pro 1 5 <210> SEQ ID NO 251 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: His box3 <400> SEQUENCE: 251 Gln Val Asp His His Leu Phe Pro 1 5 <210> SEQ ID NO 252 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: His box3 <400> SEQUENCE: 252 His Val Ala His His Leu Phe His 1 5 <210> SEQ ID NO 253 <211> LENGTH: 7 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: His box3 <400> SEQUENCE: 253 His Val Val His His Leu Phe 1 5 <210> SEQ ID NO 254 <211> LENGTH: 7 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: Corresponding amino acids to primer Phaelo forward1 <400> SEQUENCE: 254 Asn Leu Leu Trp Leu Phe Tyr 1 5 <210> SEQ ID NO 255 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: Corresponding amino acids to primer Phaelo reverse1 <400> SEQUENCE: 255 Phe Ala Gln Phe Phe Val Gln Ser 5 1

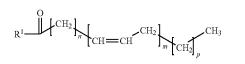
(I)

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1. A process for the production of compounds of the general formula I

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- in seeds of a transgenic plant with a content of at least 20% by weight based on the total lipid content, which comprises the following process steps:
- a) introducing, into a plant, at least one nucleic acid 15 sequence which encodes a polypeptide with $\Delta 6$ -desaturase activity.
- b) introducing, into the plant, at least one nucleic acid sequence which encodes a polypeptide with $\Delta 6$ -elon-20 gase activity.
- c) introducing, into the plant, at least one nucleic acid sequence which encodes a polypeptide with $\Delta 5$ -desaturase activity.
- d) introducing, into the plant, at least one nucleic acid gase activity, wherein said $\Delta 5$ -elongase activity elongates only unsaturated C²⁰-fatty acids, and
- e) introducing, into the plant, at least one nucleic acid sequence which encodes a polypeptide with Δ 4-desaturase activity, and
- wherein the variables and substituents in formula I have the following meanings:
- R¹=hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophospha- 35 are selected from the group consisting of: tidylserine, lysophosphatidylinositol, sphingo base or a radical of the general formula II

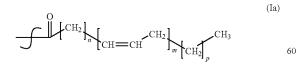
$$H_{2}C \longrightarrow O \longrightarrow R^{2}$$

$$H_{2}C \longrightarrow O \longrightarrow R^{3}$$

$$H_{2}C \longrightarrow O \longrightarrow C$$

$$(II) 40$$

- R²=hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C2-C24- 50 alkylcarbonyl,
- R³=hydrogen, saturated or unsaturated C₂-C₂₄-alkylcarbonyl, or R² and R³ independently of one another are a radical of the general formula Ia:



in which

n=2, 3, 4, 5, 6, 7 or 9, m=2, 3, 4, 5 or 6 and p=0 or 3, and wherein the at least one nucleic acid sequence which 65 encodes a polypeptide with $\Delta 5$ -elongase activity comprises:

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- i) the nucleic acid sequence of SEQ ID NO: 67, 83, or 113:
- ii) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 68, 84, or 114;
- iii) a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 67, 83, or 113: or
- iv) a nucleic acid sequence encoding an amino acid sequence having at least 95% identity to SEQ ID NO: 68, 84, or 114.
- 2. The process according to claim 1, wherein the variables n, m and p have the following meanings:
 - n=2, 3 or 5, m=4, 5 or 6 and p=0 or 3.
- 3. The process according to claim 1, wherein, in formula I, the variables n, m and p have the following meanings:
 - (a) m=4, n=3, p=3 and the compound is arachidonic acid, (b) m=5, n=3, p=0 and the compound is eicosapentaenoic acid,
- (c) m=5, n=5, p=0 and the compound is docosapentaenoic acid, or
- (d) m=6, n=3, p=0 and the compound is docosahexaenoic acid.

4. The process according to claim 2, wherein, in the seed sequence which encodes a polypeptide with $\Delta 5$ -elon- 25 of the transgenic plant, the content of all compounds of the formula I together amounts to at least 27% by weight based on the total lipid content.

> 5. The process according to claim 3, wherein, in the seed of the transgenic plant, the docosahexaenoic acid content amounts to at least 1% by weight based on the total lipid content.

> 6. The process according to claim 1, wherein the nucleic acid sequences which encode polypeptides with $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase, or $\Delta 4$ -desaturase activity

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 11, SEQ ID NO: 27, SEQ ID NO: 41, or SEQ ID NO: 193,
- b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 12, SEQ ID NO: 28, SEQ ID NO: 42, or SEQ ID NO: 194, and
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 11, SEQ ID NO: 27, SEQ ID NO: 41, or SEQ ID NO: 193, which encode polypeptides with at least 70% identity at the amino acid level with SEQ ID NO: 12, SEQ ID NO: 28, SEQ ID NO: 42, or SEQ ID NO: 194, and which have $\Delta 6$ -desaturase, $\Delta 6$ -elongase, $\Delta 5$ -desaturase or $\Delta 4$ -desaturase activity.

7. The process according to claim 1, wherein a nucleic acid sequence which encodes polypeptides with w3-desaturase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105, or
- b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 88 or SEQ ID NO: 106, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105, which encode polypeptides with at least 70% identity at the amino acid level with SEQ ID NO: 88 or SEQ ID NO: 106 and which have ω 3-desaturase activity
- is additionally introduced into the transgenic plant.
- 8. The process according to claim 1, wherein a nucleic acid sequence which encodes polypeptides with $\Delta 12$ -desaturase activity, selected from the group consisting of:

- a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 107, SEQ ID NO: 109 or SEQ ID NO: 195, or
- b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the 5 amino acid sequence shown in SEQ ID NO: 108, SEQ ID NO: 110 or SEQ ID NO: 196, or
- c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 107, SEQ ID NO: 109 or SEQ ID NO: 195, which encode polypeptides with at least 70% identity at 10 the amino acid level with SEQ ID NO: 108, SEQ ID NO: 110 or SEQ ID NO: 196 and which have Δ 12-desaturase activity
- is additionally introduced into the transgenic plant.

9. The process according to claim **1**, wherein a nucleic 15 acid sequence which encodes proteins of the biosynthetic pathway of the fatty acid or lipid metabolism selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [=acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyl- 20 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, allenoxide synthases, hydroperoxide lyases or fatty acid elon- 25 gase(s) is additionally introduced into the transgenic plant.

10. The process according to claim 1, wherein the substituents R^2 or R^3 independently of one another are saturated or unsaturated C_{18} - C_{22} -alkylcarbonyl.

11. The process according to claim 1, wherein the sub- $_{30}$ stituents R² or R³ independently of one another are unsaturated C₁₈-, C₂₀- or C₂₂-alkylcarbonyl with at least two double bonds.

12. The process according to claim **1**, wherein the transgenic plant is selected from the group consisting of an 35 oil-producing plant, a vegetable plant and an ornamental.

13. The process according to claim 1, wherein the transgenic plant is selected from the group of the plant families consisting of: Anacardiaceae, Asteraceae, Boraginaceae, Brassicaceae, Cannabaceae, Compositae, Cruciferae, Cucurbitaceae, Elaeagnaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Leguminosae, Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae, Olacaceae, Oleaceae, Papaveraceae, Piperaceae, Pedaliaceae, Poaceae and Solanaceae. 45

14. The process according to claim 1, wherein the compounds of the general formula I are isolated from the transgenic plant in the form of their oils, lipids or free fatty acids.

15. The process according to claim 1, wherein the poly- 50 peptide with Δ 5-elongase activity elongates only unsaturated C₂₀-fatty acids with one double bond in the Δ 5-position.

16. The process according to claim **1**, wherein the compounds of the general formula I comprise fatty acids having 55 20 or 22 carbon atoms in the fatty acid chain.

17. The process according to claim 1, wherein the plant is selected from the group consisting of soybean, peanut, oilseed rape, canola, linseed, evening primrose, mullein, thistle, hazelnut, almond, macadamia, avocado, bay, wild 60 roses, pumpkin/squash, pistachios, sesame, sunflower, safflower, borage, maize, poppy, mustard, hemp, castor-oil plant, olive, Calendula, *Punica*, oil palm, walnut and coconut.

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18. A process for the production of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) in seeds of a plant, comprising introducing into a plant:

- a) a nucleic acid encoding a polypeptide having $\Delta 6$ -desaturase activity;
- b) a nucleic acid encoding a polypeptide having $\Delta 6$ -elongase activity;
- c) a nucleic acid encoding a polypeptide having Δ 5-desaturase activity;
- d) a nucleic acid encoding a polypeptide having Δ 5-elongase activity; and
- e) a nucleic acid encoding a polypeptide having Δ4-desaturase activity;
- wherein said nucleic acid encoding a polypeptide having $\Delta 5$ -elongase activity comprises:
- i) the nucleotide sequence of SEQ ID NO: 67, 83, or 113;
- ii) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 68, 84, or 114; or
- iii) a nucleic acid sequence encoding a polypeptide having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 68, 84, or 114.

19. The process of claim 18, wherein said nucleic acid encoding a polypeptide having $\Delta 5$ -elongase activity comprises a nucleic acid sequence encoding a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 68, 84, or 114.

20. The process of claim **18**, wherein said nucleic acid encoding a polypeptide having $\Delta 5$ -elongase activity comprises a nucleic acid sequence encoding a polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 68, 84, or 114.

21. The process of claim **1**, wherein EPA and/or DHA is produced in the seeds of said transgenic plant.

22. A process for the production of an oil-, lipid- and fatty acid-composition, comprising:

- a) obtaining EPA and/or DHA produced by the process of claim 18; and
- b) formulating said EPA and/or DHA as an oil-, lipid- and fatty acid-composition.

23. A method for the production of feedstuffs, foodstuffs, cosmetics or pharmaceuticals, comprising:

- a) obtaining an oil-, lipid- and fatty acid-composition produced by the process of claim **22**; and
- b) processing said oil-, lipid- and fatty acid-composition to produce feedstuffs, foodstuffs, cosmetics or pharmaceuticals.

24. A process for the production of an oil-, lipid- and fatty acid-composition, comprising:

- a) producing EPA and/or DHA in seeds of a transgenic plant according to the process of claim 18; and
- b) obtaining an oil-, lipid- and fatty acid-composition from the seeds of said transgenic plant.

25. A method for the production of feedstuffs, foodstuffs, cosmetics or pharmaceuticals, comprising:

- a) obtaining an oil-, lipid- and fatty acid-composition produced by the process of claim **24**; and
- b) processing said oil-, lipid- and fatty acid-composition to produce feedstuffs, foodstuffs, cosmetics or pharmaceuticals.

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