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(71) Applicant *(for all designated States except US)*: **BASF PLANT SCIENCE COMPANY GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SENGER, Toralf** [DE/DE]; Emmendinger Weg 7, 69126 Heidelberg (DE). **MARTY, Laurent** [CH/DE]; Albert-Fritz-Str. 31, 69124 Heidelberg (DE). **STYMNE, Sten** [SE/SE]; Radmansgatan 1, S-261 32 Landskrona (SE). **LINDBERG YILMAZ, Jenny** [SE/SE]; Per Ols väg 9, S-237 37 Bfärred (SE). **NAPIER, Johnathan A.** [GB/GB]; The Wilderness, Butchers Lane, Preston, Herts AL5 2JQ (GB). **SAYANOVA, Olga** [GB/GB]; 43 Snatchup, Redbourn, St. Albans AL3 7HF (GB). **HASLAM, Richard** [GB/GB]; 77 Frederick Street, Waddesdon, Aylesbury, Buckinghamshire HP18 0LX (GB). **NOEMI, Ruiz Lopez** [ES/GB]; 3B Lawes Court, 55 Milton Road, Harpenden AL5 5NX (GB).

(74) Agent: **POPP, Andreas**; BASF SE, Global Intellectual Property, GVX/B - C 006, 67056 Ludwigshafen (DE).

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(54) **Title**: ACYLTRANSFERASES AND USES THEROF IN FATTY ACID PRODUCTION

(57) **Abstract**: The present invention relates to the recombinant manufacture of polyunsaturated fatty acids. Specifically, it relates to acyltransferase polypeptides, polynucleotides encoding said acyltransferases as well as vectors, host cells, non-human transgenic organisms containing said polynucletides. Moreover, the present invention contemplates methods for the manufacture of polyunsaturated fatty acids as well as oils obtained by such methods.

Acyltransferases and uses therof in fatty acid production

The present invention relates to the recombinant manufacture of polyunsaturated fatty acids. Specifically, it relates to acyltransferase polypeptides, polynucleotides encoding said acyltransferase polypeptides as well to vectors, host cells, non-human transgenic organisms containing said polynucletides. Moreover, the present invention contemplates methods for the manufacture of polyunsaturated fatty acids as well as oils obtained by such methods.

Fatty acids and triacylglycerides have a various applications in the food industry, in animal feed, supplement nutrition, and in the cosmetic and pharmacological and pharmaceutical field. The individual applications may either require free fatty acids or triacylglycerides. In both cases, however, polyunsaturated fatty acids either free or esterified are of pivotal interest for many of the aforementioned applications. In particular, polyunsaturated omega-3-fatty acids and omega-6-fatty acids are important constituents in animal and human food. These fatty acids are supposed to have beneficial effects on the overall health and, in particular, on the central nervous system, the cardivovascular system, the immune system, and the general metabolism. Within traditional food, the polyunsaturated omega-3-fatty acids are mainly found in fish and plant oils. However, in comparison with the needs of the industry and the need for a beneficial diet, this source is rather limited.

The various polyunsaturated fatty acids (PUFA) and PUFA-containing triglycerides are also mainly obtained from microorganisms such as Mortierella and Schizochytrium or from oil-producing plants such as soybean or oilseed rape, algae such as Crypthecodinium or Phaeodactylum and others, where they are usually obtained in the form of their triacylglycerides. The free PUFA are usually prepared from the triacylglycerides by hydrolysis. However, long chain polyunsaturated fatty acids (LCPUFA) having a C-18, C-20, C-22 or C-24 fatty acid body, such as docoahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (ARA), dihomo-gamma-linolenic acid or docosapentaenoic acid (DPA) can not be efficiently isolated from natural oil crop plants such as oilseed rape, soybean, sunflower or safflower. Conventional natural sources of these fatty acids are, thus, merely fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or from algae.

Especially suitable microorganisms for the production of PUFA in industrial scale are microalgae such as Phaeodactylum tricornutum, Porphoridium species, Thraustochytrium species, Nannochloropsis species, Schizochytrium species or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungi such as Mortierella, Entomophthora or Mucor and/or mosses such as Physcomitrella, Ceratodon and Marchantia (Vazhappilly 1998, Botanica Marina 41: 553-558; Totani 1987, Lipids 22: 1060-1062; Akimoto 1998, Appl. Biochemistry and Biotechnology 73: 269-278). Strain selection has resulted in the development of a number of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFA. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a

time-consuming and difficult process. This is why recombinant methods as described above are preferred whenever possible. However, only limited amounts of the desired PUFA or LCPUFA and, in particular, DHA or EPA, can be produced with the aid of the above mentioned microorganisms, and, depending on the microorganism used, these are generally obtained as fatty acid mixtures of, for example, EPA, DPA and DHA.

Many attempts in the past have been made to make available genes which are involved in the synthesis of fatty acids or triglycerides for the production of oils in various organisms. Various desaturases have been described in the art; see, e.g., documents WO 91/13972, WO 93/11245, WO 94/11516, EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey 1990, J. Biol. Chem., 265: 20144-20149, Wada 1990, Nature 347: 200-203, Huang 1999, Lipids 34: 649-659, WO 93/06712, US 5,614,393, WO 96/21022, WO 00/21557, WO 99/27111, WO 98/46763, WO 98/46764, WO 98/46765, WO 99/64616 or WO 98/46776. These enzymes can be used for the production of unsaturated fatty acids. Thus, due to modern molecular biology, it has become possible to increase at least to some extent the content of the desired polyunsaturated fatty acids and, in particular, the PUFA or LCPUFA in a given organism. Elongases for the production of fatty acids are disclosed in the document WO2009/016202.

The biosynthesis of LCPUFA and the incorporation of LCPUFA into membrane lipids or triacylglycerides proceeds via various metabolic pathways (Abbadi 2001, European Journal of Lipid Science & Technology 103:106-113). In bacteria such as Vibrio, and microalgae, such as Schizochytrium, malonyl-CoA is converted into LCPUFA via an LCPUFA-producing polyketide synthase (Metz 2001, Science 293: 290-293; WO 00/42195; WO 98/27203; WO 98/55625). In microalgae, such as Phaeodactylum, and mosses, such as Physcomitrella, unsaturated fatty acids such as linoleic acid or linolenic acid are converted in a plurality of desaturation and elongation steps to give LCPUFA (Zank 2000, Biochemical Society Transactions 28: 654-658). Desaturation takes place either on acyl groups bound to Coenzyme A (acyl-CoA) or on acyl groups of membrane lipids, whereas elongation is biochemicaly restricted to acyl chains bound to CoA. In mammals, the biosynthesis of DHA comprises a chain shortening via beta-oxidation, in addition to desaturation and elongation steps. In microorganisms and lower plants, LCPUFA are present either exclusively in the form of membrane lipids, as is the case in Physcomitrella and Phaeodactylum, or in membrane lipids and triacylglycerides, as is the case in Schizochytrium and Mortierella. Incorporation of LCPUFA into lipids and oils, as well as the transfer of the fatty acid moiety (acyl group) between lipids and other molecular species such as acyl-CoA, is catalyzed by various acyltransferases and transacylases. These enzymes are, known to carry out the incorporation or interexchange of saturated and unsaturated fatty acids (Slabas 2001, J. Plant Physiology 158: 505-513, Frentzen 1998, Fett/Lipid 100: 161-166, Cases 1998, Proc. Nat. Acad. Sci. USA 95: 13018-13023). One group of acyltransferases having three distinct enzymatic activities are enzymes of the "Kennedy pathway", which are located on the cytoplasmic side of the membrane system of the endoplasmic reticulum (ER). The ER-bound acyltransferases in the microsomal fraction use acyl-CoA as the activated form of fatty ac-

ids. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the incorporation of acyl groups at the sn-1 position of glycerol-3-phosphate. 1-Acylglycerol-3-phosphate acyltransferase, also known as lysophosphatidic acid acyltransferase (LPAAT), catalyze the incorporation of acyl groups at the sn-2 position of lysophosphatidic acid (LPA). After dephosphorylation of phosphatidic acid by phosphatidic acid phosphatase (PAP), diacylglycerol acyltransferase (DGAT) catalyzes the incorporation of acyl groups at the sn-3 position of diacylglycerols. Further enzymes directly involved in TAG biosynthesis – apart from the said Kennedy pathway enzymes – are the phospholipid diacylglycerol acyltransferase (PDAT), an enzyme that transfers acyl groups from the *sn*-2 position of membrane lipids to the *sn*-3 position of diacylglycerols, and diacylglyceroldiacylglycerol transacylase (DDAT), an enzyme that transfers acylgroups from the *sn*-2 position of one diacylglycerol-molecule to the *sn*-3 position of another diacylglycerol-molecule. Lysophospholipid acyltransferase (LPLAT) represents a class of acyltransferases that are capable of incorporating activated acyl groups from acyl-CoA to membrane lipids, and possibly catalyze also the reverse reaction. More specifically, LPLATs can have activity as lysophosphophatidylethanolamine acyltransferase (LPEAT) and lysophosphatidylcholine acyltransferase (LPCAT). Further enzymes, such as lecithin cholesterol acyltransferase (LCAT) can be involved in the transfer of acyl groups from membrane lipids into triacylglycerides, as well.

The documents WO 98/54302 and WO 98/54303 disclose a human LPAAT and its potential use for the therapy of diseases, as a diagnostic, and a method for identifying modulators of the human LPAAT. Moreover, a variety of acyltransferases with a wide range of enzymatic functions have been described in the documents WO 98/55632, WO 98/55631, WO 94/13814, WO 96/24674, WO 95/27791, WO 00/18889, WO 00/18889, WO 93/10241, Akermoun 2000, Biochemical Society Transactions 28: 713-715, Tumaney 1999, Biochimica et Biophysica Acta 1439: 47-56, Fraser 2000, Biochemical Society Transactions 28: 715-7718, Stymne 1984, Biochem. J. 223: 305-314, Yamashita 2001, Journal of Biological Chemistry 276: 26745-26752, and WO 00/18889.

Higher plants comprise PUFA, such as linoleic acid and linolenic acid. However, the LCPUFA ARA, EPA and DHA are not present in the seed oils of higher plants or only in traces (Ucciani: Nouveau Dictionnaire des Huiles Végétales. Technique & Documentation-Lavoisier, 1995. ISBN: 2-7430-0009-0). It is nevertheless highly desirable to produce LCPUFA in higher plants, preferably in oil seeds such as oilseed rape, linseed, sunflower and soybean, since large amounts of high-quality LCPUFA for the various aforementioned applications may be obtained thereby at low costs.

However, one drawback of using transgenic plants expressing various of the aforementioned desaturases and elongases involved in the synthesis of PUFA and LCPUFA is that the latter are not efficiently incorporated into triacylglycerides, but rather into membranes. Furthermore, efficient processing of a given acyl molecule-substrate, e.g. linoleic acid, by a plurality of desaturation and elongation steps towards the desired LCPUFA, e.g. ARA, EPA and/or DHA, is hindered by the requirement to transfer the acyl molecule and its derivatives

generated by the elongation and desaturation reactions back and forth between membrane lipids and acyl-CoA. For this reason, intermediates towards desired LCPUFA are incorporated into oil before the synthesis of the desired LCPUFA is complete. These two problems are undesired for the following reasons: First, the main lipid fraction in oil seeds are triacylglycerides. This is why, for economical reasons, it is necessary to concentrate LCPUFA in triacylglycerides. Second, LCPUFA which are incorporated into membranes can modify the physical characteristics of the membranes and thus have harmful effects on the integrity and transport characteristics of the membranes and on the stress tolerance of plants. Third, for efficient LCPUFA synthesis, it is desirable to increase the flux of intermediate-LCPUFA between the two sites of biosynthesis -that are membrane lipids and acyl-CoA - and/or decrease the flux of intermediate-PUFA/-LCPUFA into oil. Transgenic plants which comprise and express genes coding for enzymes of LCPUFA biosynthesis and produce LCPUFA have been described, e.g., in DE 102 19 203 or WO2004/087902. However, these plants produce LCPUFA in amounts which require further optimization for processing the oils present in said plants. Moreover, it was proposed that delta 6 desaturated fatty acids may be shifted into the acyl-CoA pool for increasing efficiency of fatty acid elongation in plants (Singh 2005, *Curr. Opin. Plant Biol., 8:* 197-203). Another publication demonstrated in Arabidopsis, that the additional expression of RcDGAT2 from Ricinus communis increase the storage of hydroxyfatty acids produced by a Ricinus communis fatty acid hydroxylase 12 (FAH12) from 17% to 30% in the seed oil.

Accordingly, means for increasing the content of PUFA or LCPUFA, such as EPA and DHA, in triglycerides in, e.g., plant seed oils, are still highly desirable.

Thus, the present invention relates to a polynucleotide comprising a nucleic acid sequence elected from the group consisting of:

a) a nucleic acid sequence having a nucleotide sequence as shown in any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55;

b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 53, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, and 56;

c) a nucleic acid sequence being at least 40% identical to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having acyltransferase activity;

d) a nucleic acid sequence encoding a polypeptide having acyltransferase activity and having an amino acid sequence which is at least 45% identical to the amino acid sequence of b); and

e) a nucleic acid sequence which is capable of hybridizing under one of the following sets of conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having acyltransferase activity:

f) hybridization in 50 mM Tris, pH 7.6, 6xSSC, 5xDenhardt's, 1.0% sodium dodecyl sulfat (SDS) 100µg denaturated calf thymus DNA at 34°C overnight and wash twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, repeat twice with 0.2xSSC,

0,5% SDS at room temperature for 15 min each and then repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;

g) hybridization in 6xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 0.5% SDS 100µg denaturated calf thymus DNA at 34°C overnight and wash twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, repeat twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;

h) hybridization in 20-30% formamide, 5xSSPE, 5xDenhardt's solution, 1% SDS 100µg denaturated salmon sperm DNA at 34°C overnight and wash twice with 2xSSPE, 0.2%SDS at 42°C for 15 min each, repeat twice with 2xSSPE, 0.2%SDS at 55°C for 30 min each and repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;

i) hybridization in 7% SDS, 0.5 M NaPO4, 1 mM EDTA at 50°C overnight and wash in 2 X SSC, 0.1% SDS at 50°C or 65°C;

j) hybridization in 7% SDS, 0.5 M NaPO4, 1 mM EDTA at 50°C overnight and wash in 1 X SSC, 0.1% SDS at 50°C or 65°C; or

k) hybridization in 7% SDS, 0.5 M NaPO4, 1 mM EDTA at 50°C overnight and wash in 0,1 X SSC, 0.1% SDS at 50°C or 65°C

The term "polynucleotide" as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having acyltransferase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having acyltransferas activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, LCPUFA esterified to triglycerides in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the minimal set of desaturases and elongases requiered for LCPUFA synthesis but does not express the polynucleotide of the present invention. Such a transgenic plant may, preferably, express desaturases and elongases comprised by the vector LJB765 listed in table 11 of example 5 in WO2009/016202 or a similar set of desaturases and elongases required for DHA synthesis. Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student´s t-test. More preferably, the increase is an increase of the amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, atleast 45% or at least 50% compared to the said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20, C-22 or C24 fatty acid body, more preferably, EPA or DHA, most preferably, DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples.

The term "acyltransferase activity" or "acyltransferase" as used herein encompasses all enymatic activities and enzymes which are capable of transferring or are involved in the transfer of PUFA and, in particular; LCPUFA from the acly-CoA pool or the membrane phospholipis to the triglycerides, from the acyl-CoA pool to membrane lipids and from membrane lipids to the acyl-CoA pool by a transesterification process. It will be understood

that this acyltransferase activity will result in an increase of the LCPUFA esterified to triglycerides in, e.g., seed oils. In particular, it is envisaged that these acyltransferases are capable of producing triglycerides having esterified EPA or even DHA, or that these acyltransferases are capable of enhancing synthesis of desired PUFA by increasing the flux for specific intermediates of the desired PUFA between the acyl-CoA pool (the site of elongation) and membrane lipids (the predominant site of desaturation). Specifically, acyltransferase activity as used herein relates to lysophospholipid acyltransferase (LPLAT) activity, preferably, lysophosphatidylcholine acyltransferase (LPCAT) or Lysophosphophatidylethanolamine acyltransferase (LPEAT) activity, lysophosphosphatidic acid acyltransferase (LPAAT) activity, glycerol-3-phosphate acyltransferase (GPAT) activity or diacylglycerol acyltransferase (DGAT), and, more preferably, to LPLAT, LPAAT, DGAT or GPAT activity.

More preferably, polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 1, 4, and 7, encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 2, 5, and 8 or variants thereof, preferably, exhibit LPLAT activity. Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 10, and 13, encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 11, and 14 or variants thereof, preferably, exhibit LPAAT activity. Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, and 55, encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53 , and 56 or variants thereof, preferably, exhibit DGAT activity. A polynucleotide having a nucleic acid sequence as shown in SEQ ID NO: 55, encoding a polypeptide having amino acid sequences as shown in SEQ ID NO: 56 or variants thereof, preferably, exhibit GPAT activity.

A polynucleotide encoding a polypeptide having a acyltransferase activity as specified above has been obtained in accordance with the present invention, preferably, from *Nannochloropsis oculata* and/or *Thraustochytrium aureum*. However, orthologs, paralogs or other homologs may be identified from other species.

Thus, the term “polynucleotide” as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 53, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, and 56 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide

having a acyltransferase activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled artisan and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 × sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 × SSC, 0.1% SDS at 50 to 65°C. The skilled artisan knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 6 × SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA: DNA hybrids are, preferably, 0.1 × SSC and 20°C to 45°C, preferably between 30°C and 45°C and more preferably between 45°C and 65°C. The hybridization conditions for DNA:RNA hybrids are, more preferably, 0.1 × SSC and 30°C to 55°C, most preferably between 45°C and 65°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled artisan knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 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, Oxford.

In detail variants of polynucleotides still encode a polypeptide having a acyltransferase activity as specified above comprising a nucleic acid sequence which is capable of hybridizing preferably under conditions equivalent to hybridization in 50 mM Tris, pH 7.6, 6xSSC, 5xDenhardt's, 1.0% sodium dodecyl sulfat (SDS) 100µg denaturated calf thymus DNA at 34°C overnight, followed by washing twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, then wash twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then wash twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min each to a nucleic acid described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or the complement thereof.

More preferably, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 6xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 0.5% sodium dodecyl sulfat (SDS) 100µg denaturated calf thymus DNA at 34°C overnight, followed by washing twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, then wash twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then wash twice with 0.2

SSC, 0.5% SDS at 50°C for 15 min each to a nucleic acid described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or the complement thereof.

Most preferably, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 20-30% formamide, 5xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 1% sodium dodecyl sulfat (SDS) 100µg denaturated salmon sperm DNA at 34°C overnight, followed by washing twice with 2xSSPE, 0.2%SDS at 42°C for 15 min each, then wash twice with 2xSSPE, 0.2%SDS at 55°C for 30 min each and then wash twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min each to a nucleic acid described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or the complement thereof.

In another preferred embodiment aforementioned variants of polynucleotides still encode a polypeptide having a acyltransferase activity as specified above comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4, 1 mM EDTA at 50°C overnight with washing in 2 X SSC, 0.1% SDS at 50°C or 65°C, preferably 65°C to a nucleic acid described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or the complement thereof. In still another preferred embodiment, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4, 1 mM EDTA at 50°C overnight with washing in 1 X SSC, 0.1% SDS at 50°C or 65°C, preferably 65°C to a nucleotide sequence described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or or the complement thereof, most preferably, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4, 1 mM EDTA at 50°C overnight with washing in 0,1 X SSC, 0.1% SDS at 50°C or 65°C, preferably 65°C to a nucleic acid sequence described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or the complement thereof.

The term "hybridization" as used herein includes "any process by which a strand of nucleic acid molecule joins with a complementary strand through base pairing." (J. Coombs (1994) Dictionary of Biotechnology, Stockton Press, New York). Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acid molecules) is impacted by such factors as the degree of complementarity between the nucleic acid molecules, stringency of the conditions involved, the Tm of the formed hybrid, and the G:C ratio within the nucleic acid molecules. As used herein, the term "Tm" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the Tm of nucleic acid molecules is well known in the art. As indicated by standard references, a simple estimate of the Tm value may be calculated by the

equation: Tm=81.5+0.41(% G+C), when a nucleic acid molecule is in aqueous solution at 1 M NaCl [see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985)]. Other references include more sophisticated computations, which take structural as well as sequence characteristics into account for the calculation of Tm. Stringent conditions, are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acid molecules show total complementarity to the nucleic acid molecules of the nucleic acid sequence.

The term "Complementary" or "complementarity" refers to two nucleotide sequences which comprise antiparallel nucleotide sequences capable of pairing with one another (by the base-pairing rules) upon formation of hydrogen bonds between the complementary base residues in the antiparallel nucleotide sequences. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases are not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acid molecules is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid molecule strands has significant effects on the efficiency and strength of hybridization between nucleic acid molecule strands.

Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer- based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used.

Further, variants include polynucleotides comprising nucleic acid sequences which are at least up to 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55, preferably, encoding polypeptides retaining a acyltransferase activity as specified above.

Moreover, also encompassed are polynucleotides (derivatives) which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least up to 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%,

at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the amino acid sequences shown in any one of SEQ ID NOs: 53, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, and 56, wherein the polypeptide, preferably, retains acyltransferase activity as specified above. The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled artisan for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (*EMBOSS: The European Molecular Biology Open Software Suite,* Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), *276-277, 2000)*, using either a BLOSUM 45 or PAM250 scoring matrix for distantly related proteins, or either a BLOSUM 62 or PAM160 scoring matrix for closer related proteins, and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap entension pentalty of 0.5, 1, 2, 3, 4, 5, or 6. Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at http://emboss.sourceforge.net. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the needle program in the EMBOSS software package (*EMBOSS: The European Molecular Biology Open Software Suite*, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), *276-277, 2000)*, using the EDNAFULL scoring matrix and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5,1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction for aligning two amino acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. The nucleic acid and protein sequences of the present invention can further be used as a “query sequence” to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLAST series of programs (version 2.2) of Altschul *et al.* (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using acyltransferase nucleic acid sequences of the invention as query sequence can be performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to acyltransferase sequences of the invention. BLAST using acyltransferase protein sequences of the invention as query sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences (BLASTp) or nucleic acid sequences (tBLASTn) homologous to acyltransferase sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in Altschul *et al.* (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

Table 1: Relation of sequence types of querry and hit sequences for various BLASt pro-

grams

Input query sequence	Converted Query	Algorithm	Converted Hit	Actual Database
DNA		BLASTn		DNA
PRT		BLASTp		PRT
DNA	PRT	BLASTx		PRT
PRT		tBLASTn	PRT	DNA
DNA	PRT	tBLASTx	PRT	DNA

A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a polynucleotide of the present invention. The fragment shall encode a polypeptide which still has acyltransferase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining acyltransferase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the acyltransferase activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53 , and 56 or derivative of any of these polypeptides. The activity may be tested as described in the accompanying examples.

The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Preferably, the polynucleotide of the present invention may comprise in addition to an open reading frame further untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, more preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, more preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or PUFA biosynthesis pathways, polypeptides for monitoring expression (e.g., green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. purified or at least isolated from its natural context such as its natural gene locus) or in genetically modified or exogenously (i.e. artificially) manipulated form. An isolated polynucleotide 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 nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived. The polynucleotide, preferably, is provided in the form of double or single stranded molecule. It will be understood that the present invention by referring to any of the aforementioned polynucleotides of the invention also refers to complementary or reverse complementary strands of the specific sequences or variants thereof referred to before. The polynucleotide encompasses DNA, including cDNA and genomic DNA, or RNA polynucleotides.

However, the present invention also pertains to polynucleotide variants which are derived from the polynucleotides of the present invention and are capable of interefering with the transcription or translation of the polynucleotides of the present invention. Such variant polynucleotides include anti-sense nucleic acids, ribozymes, siRNA molecules, morpholino nucleic acids (phosphorodiamidate morpholino oligos), triple-helix forming oligonucleotides, inhibitory oligonucleotides, or micro RNA molecules all of which shall specifically recognize the polynucleotide of the invention due to the presence of complementary or substantially complementary sequences. These techniques are well known to the skilled artisan. Suitable variant polynucleotides of the aforementioned kind can be readily designed based on the structure of the polynucleotides of this invention.

Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified ones such as biotinylated polynucleotides.

Advantageously, it has been found in accordance with the present invention that the polynucleotides encoding the above mentioned polypeptides having acyltransferase activity and, in particular, LPLAT, LPAAT, DGAT and/or GPAT activity, can be used for the manufacture of PUFA and, in particular, LCPUFA when expressed in a transgenic host organism or cell. Specifically, the aforementioned acyltransferase activities will allow for an increase of LCPUFA esterified to triglycerides in seed oils by shifting the said LCPUFA from the acyl-CoA pool (by polypeptides having LPAAT, DGAT or GPAT activity as specified above) and/or from the acyl-CoA pool/pospholipid pool to the phospholipid pool/acyl-CoA pool (by polypeptides having LPLAT as specified above) via transesterification. Surprisingly, it was found that the acyltransferases encoded by the polynucleotides of the present invention are also capable of efficiently shifting rather long and highly unsaturated LCPUFA towards the triglyceride pool or between the phospholipid pool and the acyl-CoA pool, in particular, even the long chain intermediates. More surprisingly even, DHA which is known to be incorporated in triglycerides only in very low amounts, if at all, can be efficiently transesterified to

triglycerides by the acyltransferases of the invention.

In particular the LPLAT of the present invention can efficiently catalyse the transesterfication of 18:2n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 18:2n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 18:2n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 18:3n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 18:3n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 18:3n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 18:3n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 18:3n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 18:3n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), transesterfication of 18:4n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 18:4n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 18:4n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 20:3n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 20:3n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 20:3n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 20:4n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 20:4n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 20:4n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 20:4n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 20:4n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 20:4n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 20:5n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 20:5n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 20:5n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 22:5n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophos-

phatidylcholine (LPC), the transesterfication of 22:5n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterfication of 22:5n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterfication of 22:6n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterfication of 22:6n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE) and/or the transesterfication of 22:6n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS).

Preferably the LPAAT of the present invention can efficiently catalyse the transesterfication of 18:2n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA), the transesterfication of 18:3n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA), the transesterfication of 18:3n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA) and/or the transesterfication of 18:4n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA).

More preferably the LPAAT of the present invention can efficiently catalyse the transesterfication of 20:3n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA), transesterfication of 20:4n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA) and/or the transesterfication of 22:5n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA).

Most preferably the LPAAT of the present invention can efficiently catalyse the transesterfication of 20:4n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA), the transesterfication of 20:5n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA) and/or the transesterfication of 22:6n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA).

Preferably the GPAT of the present invention can efficiently catalyse the transesterfication of 18:2n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P),the transesterfication of 18:3n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterfication of 18:3n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P) and/or the transesterfication of 18:4n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P).

More preferably the GPAT of the present invention can efficiently catalyse the transesterfication of 20:3n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterfication of 20:4n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P) and/or the transesterfication of 22:5n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P).

Most preferably the GPAT of the present invention can efficiently catalyse the transesterfication of 20:4n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterfication of 20:5n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P) and/or the transesterfication of 22:6n-3 from CoA to the *sn1* position of glycerole-3-phosphate

(G3P).

Preferably the DGAT of the present invention can efficiently catalyse the transesterfication of 18:2n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), transesterfication of 18:3n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), the transesterfication of 18:3n-3 from CoA to the *sn3* position of Diacylglycerol (DAG) and/or the transesterfication of 18:4n-6 from CoA to the *sn3* position of Diacylglycerol (DAG).

More preferably the DGAT of the present invention can efficiently catalyse the transesterification of 20:3n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), the transesterfication of 20:4n-3 from CoA to the *sn3* position of Diacylglycerol (DAG) and/or the transesterfication of 22:5n-3 from CoA to the *sn3* position of Diacylglycerol (DAG).

Most preferably the DGAT of the present invention can efficiently catalyse the transesterification of 20:4n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), the transesterfication of 20:5n-3 from CoA to the *sn3* position of Diacylglycerol (DAG) and/or the transesterfication of 22:6n-3 from CoA to the *sn3* position of Diacylglycerol (DAG).

The activity of the LPLAT, LPAAT, GPAT or DGAT of the present invention is useful for the specificity of a fatty acid. This fatty acid specificity is useful to generate an artificially ARA-specificity preferably. More preferably the activity of the LPLAT, LPAAT, GPAT or DGAT of the present invention is useful to generate an artificially EPA-specificity. Most preferably the activity of the LPLAT, LPAAT, GPAT or DGAT of the present invention is useful to generate an artificially DHA-specificity.

In a preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

The term “expression control sequence” as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ-PR or λ-PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy and SPO2 may be used. From yeast or fungal promoters ADC1, AOX1r, GAL1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH are, preferably, used. For animal cell or organism expression, the promoters CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer are preferably used. From

plants the promoters CaMV/35S (Franck 1980, Cell 21: 285-294], PRP1 (Ward 1993, Plant. Mol. Biol. 22), SSU, OCS, lib4, usp, STLS1, B33, nos or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0 388 186 A1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0 335 528 A1 (i.e. a abscisic-acid-inducible promoter) or WO 93/21334 (i.e. a ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0 249 676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred 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 preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from Arabidopsis, US 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 monocots: lpt-2 or lpt-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. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or LCPUFA.

The term “operatively linked” as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5´end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

In a further preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises a terminator sequence operatively linked to the nucleic

acid sequence.

The term “terminator” as used herein refers to a nucleic acid sequence which is capable of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. 2005, Plant Physiol 138, pp. 1457-1468, downstream of the nucleic acid sequence to be expressed.

The present invention also relates to a vector comprising the polynucleotide of the present invention.

The term “vector”, preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site- directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homolgous or heterologous recombination as described in detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors 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, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be men-

tioned are, in particular, various binary and co-integrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences 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 gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in E. coli and in Agrobacterium. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5, 446–451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42, 205-225.

More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called “expression cassette”) allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogene) or pSPORT1 (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith 1988, Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), 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 1988, Gene 69:301-315) and pET 11d (Studier 1990, Methods in Enzymology 185, 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 (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ-prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled artisan is familiar with other vectors which are suitable

in prokaryotic organisms; these vectors are, for example, in E. coli, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCl, in Streptomyces plJ101, plJ364, plJ702 or plJ361, in Bacillus pUB110, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667. Examples of vectors for expression in the yeast S. cerevisiae comprise pYep Sec1 (Baldari 1987, Embo J. 6:229-234), pMFa (Kurjan 1982, Cell 30:933-943), pJRY88 (Schultz 1987, Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). 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 polynucleotides of the present invention can be also 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 1983, Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow 1989, Virology 170:31-39).

The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae), see Falciatore 1999, Marine Biotechnology 1 (3):239-251 and the references cited therein, and plant cells from higher plants (for example Spermatophytes, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker 1992, Plant Mol. Biol. 20:1195-1197; Bevan 1984, Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked 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 as octopine synthase (Gielen 1984, EMBO J. 3, 835) 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 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 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 tissue-specific manner. Promoters which can be used are constitutive promoters (Benfey 1989, EMBO J. 8:2195-2202) such as those which are derived from plant viruses such as 35S CAMV (Franck 1980, Cell 21:285-

294), 19S CaMV (see US 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small subunit, which is described in US 4,962,028. Other preferred sequences for 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 Kermode 1996, Crit. Rev. Plant Sci. 15, 4: 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 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 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 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-induced PRP1-gene promoter (Ward 1993, Plant Mol. Biol. 22:361-366), the heat-inducible hsp80 promoter from tomato (US 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pinII promoter (EP 0 375 091 A). 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 promoter from oilseed rape (US 5,608,152), the USP promoter from Vicia faba (Baeumlein 1991, Mol. Gen. Genet. 225 (3):459-67), the oleosin promoter from Arabidopsis (WO 98/45461), the phaseolin promoter from Phaseolus vulgaris (US 5,504,200), the Bce4 promoter from Brassica (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein 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 promoters to be taken into consideration are the lpt2 or lpt1 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). 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.

The abovementioned vectors are only a small overview of vectors to be used in accordance with the present invention. Further vectors are known to the skilled artisan and are described, for example, in: Cloning Vectors (Ed., Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). For further suitable expression systems for prokaryotic and eukaryotic cells see the chapters 16 and 17 of Sambrook, loc cit.

It follows from the above that, preferably, said vector is an expression vector. More preferably, the said polynucleotide of the present invention is under the control of a seed-specific promoter in the vector of the present invention. A preferred seed-specific promoter as meant herein is selected from the group consisting of Conlinin 1, Conlinin 2, napin, LuFad3, USP, LeB4, Arc, Fae, ACP, LuPXR, and SBP. For details, see, e.g., US 2003-0159174.

Moreover, the present invention relates to a host cell comprising the polynucleotide or the vector of the present invention.

Preferably, said host cell is a plant cell and, more preferably, a plant cell obtained from an oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine and Soja sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacoa), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla.

Also preferably, said host cell is a microorganism. More preferably, said microorganism is a bacterium, a fungus or algae. More preferably, it is selected from the group consisting of Candida, Cryptococcus, Lipomyces, Rhodosporidium, Yarrowia and Schizochytrium.

Moreover, a host cell according to the present invention may also be an animal cell. Preferably, said animal host cell is a host cell of a fish or a cell line obtained therefrom. More preferably, the fish host cell is from herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

It will be understood that if the host cell of the invention shall be applied for LCPUFA production, it shall be capable of carrying out desaturation and elongation steps on fatty acids. To produce the LCPUFA according to the invention, the C16- or C18- fatty acids must first be 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 C18- or C20-fatty acids and after two or three elongation cycles C22- or C24-fatty acids. The activity of the desaturases and elongases used in the process according to the invention preferably leads to C18-, C20-, C22- and/or C24-fatty acids, advantageously with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds, especially preferably to give C20- and/or C22-fatty acids with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds in the molecule. After a first desaturation and the elongation have taken place, further desaturation steps such as, for example, one in the delta-5 position may take place. Products of the process according to the invention which are especially preferred are DGLA, ARA, EPA DPA and/or DHA, most preferably EPA and/or DHA. Desaturases and elongases which are required for this process may not always be present naturally in the host cell. Accordingly,

the present invention, preferably, envisages a host cell which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected organism. Preferred desaturases and/or elongases which shall be present in the host cell are at least one enzyme selected from the group consisting of: Δ-4-desaturase, Δ-5-desaturase, Δ-5-elongase, Δ-6-desaturase, Δ12-desaturase, Δ15-desaturase, ω3-desaturase and Δ-6-elongase. Especially prefered are the bifunctional d12d15-Desaturases d12d15Des(Ac) from Acanthamoeba castellanii (WO2007042510), d12d15Des(Cp) from Claviceps purpurea (WO2008006202) and d12d15Des(Lg)1 from Lottia gigantea (WO2009016202), the d12-Desaturases d12Des(Co) from Calendula officinalis (WO200185968), d12Des(Lb) from Laccaria bicolor (WO2009016202), d12Des(Mb) from Monosiga brevicollis (WO2009016202), d12Des(Mg) from Mycosphaerella graminicola (WO2009016202), d12Des(Nh) from Nectria haematococca (WO2009016202), d12Des(Ol) from Ostreococcus lucimarinus (WO2008040787), d12Des(Pb) from Phycomyces blakesleeanus (WO2009016202), d12Des(Ps) from Phytophthora sojae (WO2006100241) and d12Des(Tp) from Thalassiosira pseudonana (WO2006069710), the d15-Desaturases d15Des(Hr) from Helobdella robusta (WO2009016202), d15Des(Mc) from Microcoleus chthonoplastes (WO2009016202), d15Des(Mf) from Mycosphaerella fijiensis (WO2009016202), d15Des(Mg) from Mycosphaerella graminicola (WO2009016202) and d15Des(Nh)2 from Nectria haematococca (WO2009016202), the d4-Desaturases d4Des(Eg) from Euglena gracilis (WO2004090123), d4Des(Tc) from Thraustochytrium sp. (WO2002026946) and d4Des(Tp) from Thalassiosira pseudonana (WO2006069710), the d5-Desaturases d5Des(Ol)2 from Ostreococcus lucimarinus (WO2008040787), d5Des(Pp) from Physcomitrella patens (WO2004057001), d5Des(Pt) from Phaeodactylum tricornutum (WO2002057465), d5Des(Tc) from Thraustochytrium sp. (WO2002026946), d5Des(Tp) from Thalassiosira pseudonana (WO2006069710) and the d6-Desaturases d6Des(Cp) from Ceratodon purpureus (WO2000075341), d6Des(Ol) from Ostreococcus lucimarinus (WO2008040787), d6Des(Ot) from Ostreococcus tauri (WO2006069710), d6Des(Pf) from Primula farinosa (WO2003072784), d6Des(Pir)_BO from Pythium irregulare (WO2002026946), d6Des(Pir) from Pythium irregulare (WO2002026946), d6Des(Plu) from Primula luteola (WO2003072784), d6Des(Pp) from Physcomitrella patens (WO200102591), d6Des(Pt) from Phaeodactylum tricornutum (WO2002057465), d6Des(Pv) from Primula vialii (WO2003072784) and d6Des(Tp) from Thalassiosira pseudonana (WO2006069710), the d8-Desaturases d8Des(Ac) from Acanthamoeba castellanii (EP1790731), d8Des(Eg) from Euglena gracilis (WO200034439) and d8Des(Pm) from Perkinsus marinus (WO2007093776), the o3-Desaturases o3Des(Pi) from Phytophthora infestans (WO2005083053), o3Des(Pir) from Pythium irregulare (WO2008022963), o3Des(Pir)2 from Pythium irregulare (WO2008022963) and o3Des(Ps) from Phytophthora sojae (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)2 from Oncorhynchus mykiss (WO2005012316), d5d6Elo(Ta) from Thraustochytrium aureum (WO2005012316) and d5d6Elo(Tc) from Thraustochytrium sp. (WO2005012316), the d5-elongases d5Elo(At) from Arabidopsis thaliana (WO2005012316), d5Elo(At)2 from Arabidopsis thaliana (WO2005012316), d5Elo(Ci) from Ciona intestinalis (WO2005012316), d5Elo(Ol) from

Ostreococcus lucimarinus (WO2008040787), d5Elo(Ot) from Ostreococcus tauri (WO2005012316), d5Elo(Tp) from Thalassiosira pseudonana (WO2005012316) and d5Elo(Xl) from Xenopus laevis (WO2005012316), the d6-elongases d6Elo(Ol) from Ostreococcus lucimarinus (WO2008040787), d6Elo(Ot) from Ostreococcus tauri (WO2005012316), d6Elo(Pi) from Phytophthora infestans (WO2003064638), d6Elo(Pir) from Pythium irregulare (WO2009016208), d6Elo(Pp) from Physcomitrella patens (WO2001059128), d6Elo(Ps) from Phytophthora sojae (WO2006100241), d6Elo(Ps)2 from Phytophthora sojae (WO2006100241), d6Elo(Ps)3 from Phytophthora sojae (WO2006100241), d6Elo(Pt) from Phaeodactylum tricornutum (WO2005012316), d6Elo(Tc) from Thraustochytrium sp. (WO2005012316) and d6Elo(Tp) from Thalassiosira pseudonana (WO2005012316), the d9-elongases d9Elo(Ig) from Isochrysis galbana (WO2002077213), d9Elo(Pm) from Perkinsus marinus (WO2007093776) and d9Elo(Ro) from Rhizopus oryzae (WO2009016208).

The present invention also relates to a cell, preferably a host cell as specified above or a cell of a non-human organism specified elsewhere herein, said cell comprising a polynucleotide which is obtained from the polynucleotide of the present invention by a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination. How to carry out such modifications to a polynucleotide is well known to the skilled artisan and has been described elsewhere in this specification in detail.

The present invention furthermore relates to a method for the manufacture of a polypeptide encoded by a polynucleotide of any the present invention comprising

a) cultivating the host cell of the invention under conditions which allow for the production of said polypeptide; and
b) obtaining the polypeptide from the host cell of step a).

Suitable conditions which allow for expression of the polynucleotide of the invention comprised by the host cell depend on the host cell as well as the expression control sequence used for governing expression of the said polynucleotide. These conditions and how to select them are very well known to those skilled in the art. The expressed polypeptide may be obtained, for example, by all conventional purification techniques including affinity chromatography, size exclusion chromatography, high pressure liquid chromatography (HPLC) and precipitation techniques including antibody precipitation. It is to be understood that the method may – although preferred – not necessarily yield an essentially pure preparation of the polypeptide. It is to be understood that depending on the host cell which is used for the aforementioned method, the polypeptides produced thereby may become posttranslationally modified or processed otherwise.

The present invention encompasses a polypeptide encoded by the polynucleotide of the present invention or which is obtainable by the aforementioned method.

The term “polypeptide” as used herein encompasses essentially purified polypeptides or

polypeptide preparations comprising other proteins in addition. Further, the term also relates to the fusion proteins or polypeptide fragments being at least partially encoded by the polynucleotide of the present invention referred to above. Moreover, it includes chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications such as phosphorylation, glycosylation, myristylation and the like (Review in Mann 2003, Nat. Biotechnol. 21, 255–261, review with focus on plants in Huber 2004, Curr. Opin. Plant Biol. 7, 318-322). Currently, more than 300 posttranslational modifications are known (see full ABFRC Delta mass list at http://www.abrf.org/index.cfm/dm.home). The polypeptide of the present invention shall exhibit the acyltransferase activities referred to above.

The present invention furthermore relates to an antibody or a fragment derived thereof as an antigen which specifically recognizes a polypeptide encoded by the nucleic acid sequences of the invention.

Antibodies against the polypeptides of the invention can be prepared by well known methods using a purified polypeptide according to the invention or a suitable fragment derived therefrom as an antigen. A fragment which is suitable as an antigen may be identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either from the polypeptide of the invention by proteolytic digestion or may be a synthetic peptide. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a chimerized antibody or a fragment of any of these antibodies, such as Fab, Fv or scFv fragments etc.. Also comprised as antibodies by the present invention are bispecific antibodies, synthetic antibodies or chemically modified derivatives of any of the aforementioned antibodies. The antibody of the present invention shall specifically bind (i.e. does significantly not cross react with other polypeptides or peptides) to the polypeptide of the invention. Specific binding can be tested by various well known techniques. Antibodies or fragments thereof can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described in Köhler 1975, Nature 256, 495, and Galfré 1981, Meth. Enzymol. 73, 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be used, for example, for the immunoprecipitation, immunolocalization or purification (e.g., by affinity chromatography) of the polypeptides of the invention as well as for the monitoring of the presence of said variant polypeptides, for example, in recombinant organisms, and for the identification of proteins or compounds interacting with the proteins according to the invention.

Moreover, the present invention contemplates a non-human transgenic organism comprising the polynucleotide or the vector of the present invention.

Preferably, the non-human transgenic organism is a microorganism, more preferably the non-human transgenic organism is a fungus and most preferably the non-human transgenic

organism is a plant, plant part, or plant seed. Preferred plants to be used for introducing the polynucleotide or the vector of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Crypthecodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens, Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, 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 [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 Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis 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, 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, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var.Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the genera and species Cucurbita

maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species Phaeodactylum tricornutum, Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella, Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania, Trichodon, Skottsbergia, for example the genera and species Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus, 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 occidentalis, 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 [manihot] or Ricinus communis [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, 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 lebbek, 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], Funariaceae such as the genera Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria convoluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygrometrica

var. arctica, Funaria hygrometrica var. calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis, Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physcomitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense, 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 Linum, 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 Gossypium, 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 Elacis, for example the genus and species Elaeis guineensis [oil palm], Papaveraceae, such as the genus Papaver, for example the genera and species Papaver orientale, Papaver rhoeas, Papaver dubium [poppy], Pedaliaceae, such as the genus Sesamum, for 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, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata [cayenne pepper],

Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (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 secalinum [barley], Secale cereale [rye], Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida [oats], Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia [rice], Zea mays [maize], Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica, Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens [pepper], Capsicum annuum [paprika], Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris [tobacco], Solanum tuberosum [potato], Solanum melongena [eggplant], Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solanum lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus Camellia, for example the genus and species Camellia sinensis [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, 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 perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred plants are plants 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, or hemp.

Preferred mosses are Physcomitrella or Ceratodon. Preferred algae are Isochrysis, Mantoniella, Ostreococcus or Crypthecodinium, and algae/diatoms such as Phaeodactylum or Thraustochytrium. More preferably, said algae or mosses are selected from the group consisting of: Shewanella, Physcomitrella, Thraustochytrium, Nannochloropsis, Fusarium, Phytophthora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Phaeodactylum, Crypthecodinium, specifically from the genera and species Thallasiosira pseudonona, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Nannochloropsis oculata, Muscarioides viallii, Mortierella alpina, Phaeodactylum tricornutum or Caenorhabditis elegans or especially advantageously Phytophtora infestans and Cryptocodinium cohnii.

Transgenic plants may be obtained by transformation techniques as elsewhere in this specification. Preferably, transgenic plants can be obtained by T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). Suitable vectors are described elsewhere in the specification in detail.

Also encompassed are transgenic non-human animals comprising the vector or polynucleotide of the present invention. Preferred non-human transgenic animals envisaged by the present invention are fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

It will be understood that in order to produce the LCPUFA according to the invention, the C16- or C18- fatty acids must first be desaturated by the enzymatic activity of a desaturase and subsequently be elongated by at least two carbon atoms via an elongase in the non-human transgenic organism. After one elongation cycle, this enzyme activity gives C18- or C20-fatty acids and after two or three elongation cycles C22- or C24-fatty acids. The activity of the desaturases and elongases used in the process according to the invention preferably leads to C18-, C20-, C22- and/or C24-fatty acids, advantageously with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds, especially preferably to give C20- and/or C22-fatty acids with at least two double bonds in the fatty

acid molecule, preferably with three, four or five double bonds in the molecule. After a first desaturation and the elongation have taken place, further desaturation steps such as, for example, one in the delta-5 position may take place. Products of the process according to the invention which are especially preferred are DGLA, ARA, EPA DPA and/or DHA, most preferably EPA and/or DHA. Desaturases and elongases which are required for this process may not always be present naturally in the organism. Accordingly, the present invention, preferably, envisages a transgenic non-human organism which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected organism. Preferred desaturases and/or elongases which shall be present in the organism are at least one enzyme selected from the group consisting of: Δ-4-desaturase, Δ-5-desaturase, Δ-5-elongase, Δ-6-desaturase, Δ12-desaturase, Δ15-desaturase, ω3-desaturase and Δ-6-elongase. Especially prefered are the bifunctional d12d15-Desaturases d12d15Des(Ac) from Acanthamoeba castellanii (WO2007042510), d12d15Des(Cp) from Claviceps purpurea (WO2008006202) and d12d15Des(Lg)1 from Lottia gigantea (WO2009016202), the d12-Desaturases d12Des(Co) from Calendula officinalis (WO200185968), d12Des(Lb) from Laccaria bicolor (WO2009016202), d12Des(Mb) from Monosiga brevicollis (WO2009016202), d12Des(Mg) from Mycosphaerella graminicola (WO2009016202), d12Des(Nh) from Nectria haematococca (WO2009016202), d12Des(Ol) from Ostreococcus lucimarinus (WO2008040787), d12Des(Pb) from Phycomyces blakesleeanus (WO2009016202), d12Des(Ps) from Phytophthora sojae (WO2006100241) and d12Des(Tp) from Thalassiosira pseudonana (WO2006069710), the d15-Desaturases d15Des(Hr) from Helobdella robusta (WO2009016202), d15Des(Mc) from Microcoleus chthonoplastes (WO2009016202), d15Des(Mf) from Mycosphaerella fijiensis (WO2009016202), d15Des(Mg) from Mycosphaerella graminicola (WO2009016202) and d15Des(Nh)2 from Nectria haematococca (WO2009016202), the d4-Desaturases d4Des(Eg) from Euglena gracilis (WO2004090123), d4Des(Tc) from Thraustochytrium sp. (WO2002026946) and d4Des(Tp) from Thalassiosira pseudonana (WO2006069710), the d5-Desaturases d5Des(Ol)2 from Ostreococcus lucimarinus (WO2008040787), d5Des(Pp) from Physcomitrella patens (WO2004057001), d5Des(Pt) from Phaeodactylum tricornutum (WO2002057465), d5Des(Tc) from Thraustochytrium sp. (WO2002026946), d5Des(Tp) from Thalassiosira pseudonana (WO2006069710) and the d6-Desaturases d6Des(Cp) from Ceratodon purpureus (WO2000075341), d6Des(Ol) from Ostreococcus lucimarinus (WO2008040787), d6Des(Ot) from Ostreococcus tauri (WO2006069710), d6Des(Pf) from Primula farinosa (WO2003072784), d6Des(Pir)_BO from Pythium irregulare (WO2002026946), d6Des(Pir) from Pythium irregulare (WO2002026946), d6Des(Plu) from Primula luteola (WO2003072784), d6Des(Pp) from Physcomitrella patens (WO200102591), d6Des(Pt) from Phaeodactylum tricornutum (WO2002057465), d6Des(Pv) from Primula vialii (WO2003072784) and d6Des(Tp) from Thalassiosira pseudonana (WO2006069710), the d8-Desaturases d8Des(Ac) from Acanthamoeba castellanii (EP1790731), d8Des(Eg) from Euglena gracilis (WO200034439) and d8Des(Pm) from Perkinsus marinus (WO2007093776), the o3-Desaturases o3Des(Pi) from Phytophthora infestans (WO2005083053), o3Des(Pir) from Pythium irregulare (WO2008022963), o3Des(Pir)2 from

Pythium irregulare (WO2008022963) and o3Des(Ps) from Phytophthora sojae (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)2 from Oncorhynchus mykiss (WO2005012316), d5d6Elo(Ta) from Thraustochytrium aureum (WO2005012316) and d5d6Elo(Tc) from Thraustochytrium sp. (WO2005012316), the d5-elongases d5Elo(At) from Arabidopsis thaliana (WO2005012316), d5Elo(At)2 from Arabidopsis thaliana (WO2005012316), d5Elo(Ci) from Ciona intestinalis (WO2005012316), d5Elo(Ol) from Ostreococcus lucimarinus (WO2008040787), d5Elo(Ot) from Ostreococcus tauri (WO2005012316), d5Elo(Tp) from Thalassiosira pseudonana (WO2005012316) and d5Elo(Xl) from Xenopus laevis (WO2005012316), the d6-elongases d6Elo(Ol) from Ostreococcus lucimarinus (WO2008040787), d6Elo(Ot) from Ostreococcus tauri (WO2005012316), d6Elo(Pi) from Phytophthora infestans (WO2003064638), d6Elo(Pir) from Pythium irregulare (WO2009016208), d6Elo(Pp) from Physcomitrella patens (WO2001059128), d6Elo(Ps) from Phytophthora sojae (WO2006100241), d6Elo(Ps)2 from Phytophthora sojae (WO2006100241), d6Elo(Ps)3 from Phytophthora sojae (WO2006100241), d6Elo(Pt) from Phaeodactylum tricornutum (WO2005012316), d6Elo(Tc) from Thraustochytrium sp. (WO2005012316) and d6Elo(Tp) from Thalassiosira pseudonana (WO2005012316), the d9-elongases d9Elo(Ig) from Isochrysis galbana (WO2002077213), d9Elo(Pm) from Perkinsus marinus (WO2007093776) and d9Elo(Ro) from Rhizopus oryzae (WO2009016208).

Furthermore, the present invention encompasses a method for the manufacture of polyunsaturated fatty acids comprising:

a) cultivating the host cell of the invention under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
b) obtaining said polyunsaturated fatty acids from the said host cell.

The term “polyunsaturated fatty acids (PUFA)” as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term polyunsaturated fatty acids relates to long chain PUFA (LCPUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19) and, more preferably, from EPA and DHA. Thus, it will be understood that most preferably, the methods provided by the present invention relating to the manufacture of EPA or DHA. Moreover, also encompassed are the intermediates of LCPUFA which occur during synthesis starting from oleic acid 18:1 (9), preferably, linoleic acid 18:2 (9,12), alpha-linolenic acid 18:3 (9,12,15), gamma-linolenic acid 18:3 (6,9,12), stearidonic acid 18:4 (6,9,12,15), dihomo-gamma-linoleic acid 20:3 (8,11,14), eicosadienoic acid 20:2 (11,14), eicosatrienoic acid 20:3 (11,14,17), eicosatetraenoic acid 20:4 (8,11,14,17) and docospentaenoic acid (DPA) 22:5 (4,7,10,13,16).

The term “cultivating” as used herein refers maintaining and growing the host cells under

culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or LCPUFA referred to above, preferably, as triglyceride esters. This implies that the polynucleotide of the present invention is expressed in the host cell so that the acyltransferase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

The term “obtaining” as used herein encompasses the provision of the cell culture including the host cells and the culture medium as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, as triglyceride esters. More preferably, the PUFA and LCPUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

The host cells to be used in the method of the invention are grown or cultured in the manner with which the skilled artisan is familiar, depending on the host organism. Usually, host cells are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0°C and 100°C, preferably between 10°C and 60°C under oxygen or anaerobic atmosphere depedent on the type of organism. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The cultures can be grown batchwise, semibatchwise or continuously. Nutrients can be provided at the beginning of the fermentation or administerd semicontinuously or continuously: The produced PUFA or LCPUFA can be isolated from the host cells as described above by processes known to the skilled artisan, e.g., by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. It might be required to disrupt the host cells prior to purification. To this end, the host cells can be disrupted beforehand. The culture medium to be used must suitably meet the requirements of the host cells in question. Descriptions of culture media for various microorganisms which can be used as host cells according to the present invention can be found in the textbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Culture media can also be obtained from various commercial suppliers. All media components are sterilized, either by heat or by filter sterilization. All media components may be present at the start of the cultivation or added continuously or batchwise, as desired. If the polynucleotide or vector of the invention which has been introduced in the host cell further comprises an expressible selection marker, such as an antibiotic resistance gene, it might be necessary to add a selection agent to the culture, such as an antibiotic in order to maintain the stability of the introduced polynucleotide. The culture is continued until formation of the desired product is at a maximum. This is normally achieved within 10 to 160 hours. The fermentation broths can be used directly or can be processed further. The biomass may, according to requirement, be removed completely or partially from the fermentation broth by separation methods such as, for example, centrifugation, filtration, decanting or a combination of these methods or be left

completely in said broth. The fatty acid preparations obtained by the method of the invention, e.g., oils, comprising the desired PUFA or LCPUFA as triglyceride esters are also suitable as starting material for the chemical synthesis of further products of interest. For example, they can be used in combination with one another or alone for the preparation of pharmaceutical or cosmetic compositions, foodstuffs, or animal feeds. Chemically pure triglycerides comprising the desired PUFA or LCPUFA can also be manufactured by the methods described above. To this end, the fatty acid preparations are further purified by extraction, distillation, crystallization, chromatography or combinations of these methods. In order to release the fatty acid moieties from the triglycerides, hydrolysis may be also required. The said chemically pure triglycerides or free fatty acids are, in particular, suitable for applications in the food industry or for cosmetic and pharmacological compositions.

Moreover, the present invention relates to a method for the manufacture of polyunsaturated fatty acids comprising:

a) cultivating the non-human transgenic organism of the invention under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
b) obtaining said polyunsaturated fatty acids from the said non-human transgenic organism.

Further, it follows from the above that a method for the manufacture of an oil, lipid or fatty acid composition is also envisaged by the present invention comprising the steps of any one of the aforementioned methods and the further step of formulating PUFA or LCPUFA as oil, lipid or fatty acid composition. Preferably, said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or pharmaceuticals. Accordingly, the formulation of the PUFA or LCPUFA shall be carried out according to the GMP standards for the individual envisaged products. For example, oil may be obtained from plant seeds by an oil mill. However, for product safety reasons, sterilization may be required under the applicable GMP standard. Similar standards will apply for lipid or fatty acid compositions to be applied in cosmetic or pharmaceutical compositions. All these measures for formulating oil, lipid or fatty acid compositions as products are comprised by the aforementioned manufacture.

The present invention also relates to oil comprising a polyunsaturated fatty acid or a polyunsaturated fatty acid composition obtainable by the aforementioned methods.

The term “oil” refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or LCPUFA as referred to above. The amount of esterified PUFA and/or LCPUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and LCPUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-

naturally occurring composition with respect to the PUFA and/or LCPUFA composition and content. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and LCPUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

The contents of all references cited throughout this application are herewith incorporated by reference in general and with respect to their specific disclosure content referred to above.

This invention is further illustrated by the following figures and examples which should not be construed as limiting the scope of the invention.

FIGURES

Figure 1: Cloning strategy employed for stepwise buildup of plant expression plasmids of the invention.

Figure 2: Vector map of the bbc construct used for Arabidopsis transformation.

Figure 3: GC chromatogram of fatty acids methyl esters of total fatty acids of Col-0, *fae1* mutant and *fae1* transformed with bbc. Total fatty acids were measured as descibed by Wu et al., 2005. The content of the different fatty is indicated in table 5.

Figure 4: Total ion count of 26 acyl CoA ESI-MS/MS MRM pairs for Arabidopsis (A) Col-0 and (B) *fae1* harbouring EPA biosynthesis pathway. Maturing Arabidopsis seeds were harvested 18 days after flowering. Acyl-CoA was extracted according to Larson et al (2001) and LC conditions after Han et al. (2010).

Figure 5: Identification of Eicosapentaenoic and Arachidonic-CoA's in the acyl CoA pool of Arabidopsis Col-0 and EPA producing plants. MRM chromatograms of co-eluting acyl-CoA of interest in (A) wild type and (C) *fae1* harbouring EPA biosynthetic pathway with recorded reactions shown for each transition, isotopic peaks (IP) of homologous long chain acyl CoA are shown. (B) Characteristic fragmentation of the protonated acyl-CoA by neutral loss of 507 to give the protonated acyl pantetheine group.

Figure 6: LPCAT activity assay.
A yeast mutant lacking LPEAT and LPCAT activity (due to knockout of the gene YOR175c) was transformed with the empty vector pYES2.1 (lane marked "–") and with pYES2.1 harboring the cDNA of pLPAAT_c6316(No) (lane 1 and 2, SEQ-ID: 13). Microsomal isolations of these transformants and the wildtype yeast strain BY4742 (lane marked "+") containing 5µg protein where incubated with alpha-linolenic acid-CoA and [^{14}C]-18:1-

lysophosphatidylcholine (LPC). Thin layer chromatography was performed to separate lipid classes. Like for wildtype yeast (lane marked "+"), phosphatidylcholine (PC) is observed for both yeast clones shown in lane 1 and 2, indicating the gene pLPAAT_c6316(No) has LPCAT activity and complements the missing LPCAT activity of the knockout strain.

Figure 7: LPAAT activity assay.
A yeast mutant lacking LPAAT activity (due to knockout of the gene YDL052c) was transformed with the empty vector pYES2.1 (lane marked "–") and with pYES2.1 harboring the cDNA of pLPAAT_c6316(No) (lane 1 and 2, SEQ-ID: 13). Microsomal isolations of these transformants and the wildtype yeast strain BY4742 (lane marked "+") containing 5µg protein where incubated with alpha-linolenic acid-CoA and [^{14}C]-18:1-lysophosphatidic acid (LPA). Thin layer chromatography was performed to separate lipid classes. Like for wildtype yeast (lane marked "+"), phosphatidic acid (PA) is observed for both yeast clones shown in lane 1 and 2, indicating the gene pLPAAT_c6316(No) has LPAAT activity and complements the missing LPAAT activity of the knockout strain.

Figure 8: DGAT activity assay.
A yeast mutant lacking the capability to synthesis TAG (due to knockout of the four genes YCR048W, YNR019W, YOR245C and YNR008W) was transformed with the empty vector pYES2.1 (lane marked "–") and with pYES2.1 harboring the cDNA of pDGAT2-c19425mod(Ta) (SEQ-ID 52, lane 1 and 2), pDGAT2_c4648(No) (SEQ-ID 34, lane 5 and 6), pDGAT2_c48271(No) (SEQ-ID 102, lane 7 and 8), BnDGAT1 (SEQ-ID 107, lane 9 and 10), AtDGAT1 (SEQ-ID 105, lane 11 and 12), pDGAT2_c699(No) (SEQ-ID 19, lane 13 and 14) and pDGAT2_c2959(No) (SEQ-ID 25, lane 15). Microsomal isolations of these transformants and the wildtype yeast strain G175 (lane marked "+") where incubated with ^{14}C-labled oleic acid and diacylglyerole (DAG). Thin layer chromatography was performed to separate lipid classes. Like for wildtype yeast (lane marked "+"), triacylglycerole (TAG) is observed in lane 1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, indicating pDGAT2-c19425mod(Ta), pDGAT2_c4648(No), pDGAT2_c48271(No), BnDGAT1, AtDGAT1, pDGAT2_c699(No) and pDGAT2_c2959(No) encode polypeptides having DGAT activity and complement the missing TAG-synthesis capability of the knockout.

Figure 9: Substrate specificity of AtDGAT1 and BnDGAT1. The specific activity of the enzymes AtDGAT1 and BnDGAT1 using the substrates indicated at the x-axis is given as the amount (in nmol) of substrate consumed in one minute per mg total protein and was determined as described in example 10.

Figure 10: Substrate specificity of pDGAT2-c19425(Ta) compared to AtDGAT1 and BnDGAT1. The specific activity of the enzymes pDGAT2-c19425(Ta), AtDGAT1 and BnDGAT1 using the substrates indicated at the x-axis is given as the amount (in nmol) of substrate consumed in one minute per mg total protein and was determined as described in example 10.

Figure 11: Substrate specificity of pDGAT2_c699(No) and pDGAT2_c4648(No) compared to AtDGAT1 and BnDGAT1. The specific activity of the enzymes pDGAT2_c699(No) and pDGAT2_c4648(No), AtDGAT1 and BnDGAT1 using the substrates indicated at the x-axis is given as the amount (in nmol) of substrate consumed in one minute per mg total protein and was determined as described in example 10.

EXAMPLES

Example 1: General cloning methods

Cloning methods as e.g. use of restriction endonucleases to cut double stranded DNA at specific sites, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic aicds onto nitrocellulose and nylon memebranes, joining of DNA-fragments, transformation of E.coli cells and culture of bacteria where perforemed as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87965-309-6).

Example 2: Sequence Analysis of recombinant DNA

Sequencing of recombinant DNA-molecules was performed using a laser-fluorescence DNA sequencer (Applied Biosystems Inc, USA) employing the sanger method (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467). Expression constructs harboring fragments obtained by polymerase chain reactions were subjected to sequencing to confirm the correctness of expression cassettes consisting of promoter, nulceic acid molecule to be expressed and terminator to avoid mutations that might result from handling of the DNA during cloning, e.g. due to incorrect primers, mutations from exposure to UV-light or errors of polymerases.

Example 3: Cloning of yeast expression construct via homologous recombination

The open reading frame listed in SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 55, 102, 105 and 107 encoding polypeptides with the amino acid sequence SEQ ID NOs: 53, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 56 ,103, 106 and 108 that have acyltransferase activity can amplified using the primer listed in table 2 in a polymerase chain reaction. By doing so, the open reading frame is 5' fused to about 60 nucleotides of the 3' end of the GAL1 promotor sequence with simultanious introduction of and *Asc* I and/or *Nco* I restriction site between the fusion site and 3' fused to about 60 nucleotides of the 5' end of the CYC1 terminator sequence with simultanious introduction of and *Pac* I restriction site. To integrate these fragments into pYES2.1 TOPO downstream of the galactose inducible GAL1 Promotor via homologous recombination, the vector pYES2.1 (Invitrogen) can be digested using the restriction endonucleases *Pvu* II and *Xba* I, and Saccharomyces cerevisea can be transformed with 5 to 20ng of linearized pYES2.1 TOPO vector and 20 to 100ng PCR product per 50 µl competent cells using the transformation method described by Schiestl et al. (Schiestl et al. (1989) Curr. Genet. 16(5-

6), pp. 339-346), to obtain pYES-pLPLAT_c1216(No), pYES-pLPLAT_c3052(No), pYES-pLPEAT-c7109(Ta), pYES-pLPAAT_c2283(No), pYES-pLPAAT_c6316(No), pYES-pDGAT2_lrc24907(No), pYES-pDGAT2_c699(No), pYES-pDGAT2_c1910(No), pYES-pDGAT2_c2959(No), pYES-pDGAT2_c4857(No), pYES-pDGAT1_c21701(No), pYES-pDGAT2_c4648(No), pYES-pDGAT2_c1660(No), pYES-pDGAT2_c29432(No), pYES-pDGAT2_c1052(No), pYES-pDGAT2-c18182(Ta), pYES-pDGAT2-c5568(Ta), pYES-pDGAT2-c19425(Ta), pYES-pDGAT2_c48271(No), AtDGAT1, BnDGAT1 and pYES-pGPAT_c813(No) in various wildtype yeasts and yeast mutants. Positive transformants can be selected based on the complementation of the URA auxotrophy of the chosen S.cerevisia strain. To validate the correctness of the expression construct harbored by a particular yeast clone, plasmids can be isolated as described in Current Protocols in Molecular Biology (Hoffmann, Curr. Protoc. Mol. Biol. 2001 May; Chapter 13:Unit13.11), transformed into E.coli for amplification and subjected to sequencing of the expression cassette as described in example 2. For later cloning into plant expression plasmids, the introduced restrictions site for *Asc* I and/or *Nco* I in combination with *Pac* I can be used.

Table 2: Primer sequences for cloning acyltransferase-polynucleotides of the invention for yeast expression

Gene-Name	Primer	SEQ-ID
pLPLAT_c1216(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatggacaaggcactggcaccgtt	46
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaactaaactttcttccttccctcta	47
pLPLAT_c3052(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatgaccacgactgtcatctctag	48
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaatcaaagcctcccgcacaacgagc	49
pLPEAT-c7109(Ta)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatggagggcatcgagtcgatagt	50
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaactataaggcttctcccggcgcgg	51

Gene-Name	Primer	SEQ-ID
pLPAAT_c2283(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgaa-gacgcccacgagcctggc	52
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaattaagctctc-gaatcgtccttct	53
pLPAAT_c6316(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatggtcag-gaggaagatggacgt	54
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaatcac-gacgccggcgccttgcagt	55
pD-GAT2_lrc24907(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgg-caccctccccaccggcccc	56
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaatcatttgaccac-taaggtggcct	57
pDGAT2_c699(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgggtc-tatttggcagcgggat	58
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactaaaagaaatt-caacgtccgat	59
pDGAT2_c1910(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgttgag-tatccccgagtcgtc	60
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactaaaagaaatc-agctccctgt	61

Gene-Name	Primer	SEQ-ID
pDGAT2_c2959(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccat-gacgccgcaagccgatatcac	62
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaattactcaatgga-caacgggcgcg	63
pDGAT2_c4857(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatggct-tacctcttccgtcgtcg	64
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaattaggcgatcgcaat-gaactcct	65
pDGAT1_c21701(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccac-catgccttttggacgggctgcatc	66
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaatcacccgaaaa-tatcctccttct	67
pDGAT2_c4648(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatggc-caaggctaacttcccgcc	68
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaatcactttataag-cagcttcttgt	69
pDGAT2_c1660(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatgttgttg-cagggattaagctg	70
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactccttccttttcggttagagcggatttaattaatcacaacaggac-caatttatgat	71

Gene-Name	Primer	SEQ-ID
pDGAT2_c29432(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgtt-gatggcgccgtcgcggcg	72
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaatcagacgatgc-gaagcgtcttgt	73
pDGAT2_c1052(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgggcgc-taccactgcgaccca	74
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaatcacgacttcgga-cagtccaaaa	75
pDGAT2-c18182(Ta)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccac-catgtcgttcgttgagcacagcgc	76
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactacacaaatcg-catcgtcttgt	77
pDGAT2-c5568(Ta)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccac-catggtcttcctctgccttcccta	78
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactacgagtccagc-cacttgatgc	79
pDGAT2-c19425(Ta)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccac-catgtttcttcgcatcgaacggga	80
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactaaccctcggtgta-cagcgccg	81

Gene-Name	Primer	SEQ-ID
pGPAT_c813(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgc-catcccgcagcaccattga	82
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaatcaga-caagctcctcttcccct	83
pDGAT2_c48271(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatggccgc-catctcaccgcgcaa	109
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactaccacacctc-caacttcgccc	110
AtDGAT1	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatggc-gattttggattctgctgg	111
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaatcatgacatc-gatccttttcggt	112
BnDGAT1	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgt caaggagaaaaaaccccggatcggcgcgccaccatgga-gattttggattctggagg	113
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaact ccttccttttcggttagagcggatttaattaactatga-catctttcctttgcggt	114

Table 3: Coding polynucleotide sequences, amino acid sequences encoded thereby and expressed sequences (mRNA) of the acyltransferases of the invention

Gene name	Organism	ORF in bp	SEQ-ID No.	Ami-no acids	SEQ-ID No.	mRNA in bp	SEQ-ID No.
pLPLAT_c1216(No)	Nannochlo-ropsis oculata	1485	1	494	2	1908	3
pLPLAT_c3052(No)	Nannochlo-ropsis oculata	1776	4	591	5	2247	6

pLPEAT-c7109(Ta)	Thraustochytrium aureum	1134	7	377	8	1288	9
pLPAAT_c2283(No)	Nannochloropsis oculata	1284	10	427	11	1826	12
pLPAAT_c6316(No)	Nannochloropsis oculata	1395	13	464	14	1771	15
pDGAT2_lrc24907(No)	Nannochloropsis oculata	1026	16	341	17	1100	18
pDGAT2_c699(No)	Nannochloropsis oculata	1206	19	401	20	1772	21
pDGAT2_c1910(No)	Nannochloropsis oculata	1173	22	390	23	1239	24
pDGAT2_c2959(No)	Nannochloropsis oculata	1089	25	362	26	1609	27
pDGAT2_c4857(No)	Nannochloropsis oculata	1464	28	487	29	1682	30
pDGAT1_c21701(No)	Nannochloropsis oculata	1539	31	512	32	1904	33
pDGAT2_c4648(No)	Nannochloropsis oculata	1083	34	360	35	1362	36
pDGAT2_c1660(No)	Nannochloropsis oculata	1695	37	564	38	2074	39
pDGAT2_c29432(No)	Nannochloropsis oculata	1029	40	342	41	1585	42
pDGAT2_c1052(No)	Nannochloropsis oculata	1251	43	416	44	1923	45
pDGAT2-c18182(Ta)	Thraustochytrium aureum	930	46	309	47	1134	48
pDGAT2-c5568(Ta)	Thraustochytrium aureum	1179	49	392	50	1303	51
pDGAT2-c19425(Ta)	Thraustochytrium aureum	1389	52	462	53	1547	54
pGPAT_c813(No)	Nannochloropsis oculata	1977	55	658	56	2460	57
pDGAT2_c48271(No)	Nannochloropsis oculata	960	102	319	103	1265	104

Example 4: Assembly of genes required for PUFA synthesis within a T-plasmid

For synthesis of EPA in Arabidopsis seeds, the set of genes encoding the proteins of the metabolic EPA pathway (table 4) was combined with expression elements (promoter, terminators) and transferred into binary t-plasmids that were used for agrobacteria mediated transformation of plants as described in example 5. To this end, the general cloning strategy depicted in figure 1 was employed: Genes listed in table 4 were PCR-amplified using Phusion™ High-Fidelity DNA Polymerase (NEB, Frankfurt, Germany) according to the manufactures instructions from cDNA using primer introducing a *Nco* I and/or *Asc* I restriction site at the 5' terminus, and a *Pac* I restriction site at the 3' terminus (figure 1B). To obtain the final expression modules, PCR-amplified genes were cloned between promoter and terminator via Nco I and/or Pac I restriction sites (figure 1C). Up to three of those expression modules were combined as desired to expression cassettes harbored by either one of pENTR/A, pENTR/B or pENTR/C (figure 1D).. Finally, the Multisite Gateway™ System (Invitrogen) was used to combine three expression cassette harbored by pENTR/A, pENTR/B and pENTR/C (figure 1E) to obtain the final binary T-plasmids bbc (SEQ-ID 101, figure 2).

Table 4: Genes of the bbc construct for synthesis of EPA (20:5n-3) in Arabidopsis seeds. The elements controlling the expression of the respective genes are as well indicated.

Name	Source Organism	Activity	SEQ-ID	Promoter	Terminator
d12Des(Ps)	*Phytophtora sojae*	d-12 Desaturase	96	p-BnNapin	t-E9
d6Des(Ot)	*Ostreococcus tauri*	d-6 Desaturase	97	p-SBP	t-CatpA
d5Des(Tc)	*Traustochytrium ssp.*	d-5 Desaturase	98	p-LuCnl	t-AgroOCS
d6Elo(Pp)	*Physcomitrella patens*	d-6 Elongase	99	p-VfUSP	t-CaMV35S
o-3Des(Pi)	Phytophthora infestans	o-3 Desaturase	100	p-Napin	t-E9

Example 5: Plant Transformation

The resulting binary vector bbc harboring the genes reconstituting EPA biosynthesis pathway were transformed into Agrobacterium tumefaciens (Hofgen and Willmitzer (1988) Nucl. Acids Res. 16: 9877). The transformation of A. thaliana was accomplished by means of the floral-dip method (Clough and Bent (1998) Plant Journal 16: 735-743), this method is known to the skilled person. Wild-type Arabidopsis seeds contain considerable amounts of eicosenoic acid (20:1) (Table 5). Biosynthesis of 20:1 competes for the substrates of the PUFA biosynthesis pathway. This competition was circumvented by transforming bbc into the Arabidopsis fae1 mutant (James et al., (1995) The Plant Cell 7:309-319).

Example 6: Quantification of metabolic fatty Acyl-CoAs in wild-type and EPA producing Arabidopsis seeds

The selected transgenic Arabidopsis plants from example 3 were analyzed in respect to PUFA content in seeds. Seeds from wild-type, fae1 mutant and transgenics harboring the

bbc construct were harvested 18 days after flowering. Total fatty acid, representing the fatty acids esterified to CoA, on lipids and as triacyl-glycerides were extracted and analyzed by gas chromatography as described in Wu et al., (2005) Nature Biotechnology 23(8): 1013-1017.

In seeds of fae1 transformed with bbc the EPA accumulation was 12.2 %, the seeds contained small amounts of indermediate or side products: ARA (3.2 %), SDA (0.8 %), GLA (2.6 %) which were not present in wild-type or fae1 (Fig 3, Table 5).

Table 5: Content of fatty acids in seeds of wild-type (Col-0), fae1 mutant and fae1 transformed with bbc construct

Fatty acid	Common name of FA	Col-0	*fae1*	bbc *fae1*
16:0	Palmitic acid	6,2	8,8	6,8
18:0	Stearic acid	3,1	4,1	5,3
18:1	Oleic acid	16,3	27,5	18,9
18:2	Linoleic acid	28,2	39,0	30,8
18:3n6	Gamma-Linolenic acid	0,0	0,0	2,6
18:3n3	Alpha-Linoleic acid	15,6	18,4	11,9
18:4n3	Stearidonic acid	0,0	0,0	0,8
20:1	Eicosenoic acid	22,8	0,4	0,3
20:4n6	Arachidonic acid	0,0	0,0	3,2
20:5n3	Eicosapentaenoic acid	0,0	0,0	12,2
Others		7,8	1,8	7,2

For PUFA biosynthesis the acyl-moiety has to be shuffled between different metabolic pools. For example, the elongation of the acyl chain by two carbon atoms occurs specifically on acyl-CoA (Zank et al., (2002) The Plant Journal 318(3):255-268. The efficency of the transfer of the acyl-residue between the metabolic pools may represent a bottleneck for PUFA production in plants. Therefore the accumulation of EPA or intermediates of EPA biosynthesis as CoA species was analyzed by LC/MS^2. As a control CoA pool of wild-type seeds were as well analyzed. The Acyl-CoA metabolites were extraced from the seed tissue according to Larson and Graham, 2001. LC/MS^2 was applied as described by Magnes et al., 2005. Briefly, CoA were separeted with high resolution by reversed-phase high performance liquid chromatography (HPLC) with a ammonium hydroxide and acetonitrile gradient. The acyl-CoA species were identified and quantified by multireaction monitoring using triple quadrupole mass spectrometry. Only a few methods using mass spectrometry for characterization of long chain acyl-CoA have been published, the majority of which employ negative ionisation mode showing abundant ions. In contrast, positive ionisation has only one abundant ion [M – H]+, furthermore the predominant ion in MS^2 spectra is the fatty acyl-pantetheine fragment (m/z 507 - Fig 5 B), characteristic of CoA-activated substances. In choosing the acyl-pantetheine of interest in multireaction monitoring mode (MRM) a very sensitive, selective and reproducible method was established. CoA-activated substances can be monitored by scanning for the neutral loss of phosphoadenosine diphosphate. Generally for reliable analysis, all interfering peaks must

be chromatographically separated; in the case of EPA and ARA this is not possible (Fig 4 B). However through the use of MRM, incorporating very short dwell times (15 ms), it is possible to follow the individual chromatograms of acyl-CoA of interest and demonstrate the presence of EPA and ARA in the acyl CoA pool (Fig 5 C). According to internal standards the eicosapentaenoyl-CoA was in the range of …% of the total Co-A pool.

In conclusion these results show that PUFA accumulate in the metabolic CoA pool and are not transferred to DAG to be released as TAG into the seed oil. Such a bottleneck may be overcome by the co-expression of an acyltransferase from table 3, having the appropriate substrate specificity. The application of suitable acyltransferase may increase the flux of fatty acid between the metabolic pools and increase the PUFA biosynthesis rate.

Example 7: Activity assays using yeast extracts

To characterize the functions of the acyltransferase polypeptides of the invention, yeast mutants can be employed that are defective in certain acyltransferase activities. For example, the yeast mutant Y13749 (Genotype: BY4742; Mat alpha; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YDL052c::kanMX4) lacking LPAAT activity can be transformed with expression constructs harboring candidate polypeptides to check for restoration (complementation) of LPAAT activity, the yeast mutant Y12431 (genotype BY4742; Mat alpha; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YOR175c::kanMX4) lacking LPLAT activity can be transformed with expression constructs harboring candidate polypeptides to check for restoration (complementation) of LPLAT activity, the yeast mutant H1246 (genotype MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 YOR245::KanMX4 YNR008W::TRP1 YCR048W::HIS3 YNR019W::LEU2) lacking the ability to synthesize triacylglycerole can be transformed with expression constructs harboring candidate polypeptides to check for restoration (complementation) of the ability to synthesis triacylglycerole. The yeast mutants can for example harbor the expression constructs listed in example 3 employing the transformation method described in example 3.

For LPAAT activity assay, clones of the yeast mutant Y13749 harboring pYES-pLPAAT_c6316(No) can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptide can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml resuspention buffer (25 mM Tris/HCL pH 7.6) and disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant is transferred to a fresh tube and centrifuged at 3000 x g for 5 min. The obtained supernatant is the crude extract. Protein content is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 1 to 50 µg of protein, 10 µl of 100 nM [^{14}C]-18:1-LPA (giving about 2000 dpm/nmol), 10 µl of 50 nM 18:1-CoA or 50nM 18:3n-3-CoA in assay buffer (25mM Tris/HCL pH 7.6, 0.5 mg/ml

BSA) to give a total volume of 100 µl. Samples are incubated for 10 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Blight and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). It can be seen by the formation of phosphatidic acid (PA) in figure 7, that pLPAAT_c6316(No) (SEQ-ID 13, lane 1 and 2) encodes a polypetide having LPAAT activity.

For LPCAT and LPEAT activity assay, clones of the yeast mutant Y12431 harboring pYES-pLPAAT_c6316(No) can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptide can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspendet in 1 ml resuspention buffer (25 mM Tris/HCL pH 7.6) and disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant is transferred to a fresh tube and centrifuged at 3000 x g for 5 min. The obtained supernatant is the crude extract. Protein content is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain either 10µl 100nM [^{14}C]-LPC (LPCAT activity assay) or 10µl 100nM [^{14}C]-LPE (LPEAT activity assay), 1 to 50 µg of protein, 10 µl of 50nM 18:1-CoA or 50nM 18:3n-3-CoA in assay buffer (25mM Tris/HCL pH 7.6, 0.5 mg/ml BSA) to give a total volume of 100 µl. Samples are incubated for 10 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Blight and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). It can be seen by the formation of phosphatidylethanolamine (PC) in figure 6, that pLPAAT_c6316(No) (SEQ-ID 13, lane 1 and 2) encodes a polypeptide having LPCAT activity.

For DGAT activity assay, clones of the yeast mutant H1246 harboring either one of pYES-pDGAT2_c699(No), pYES-pDGAT2_c2959(No), pYES-pDGAT2_c4648(No), pYES-pDGAT2_c48271(No), pYES-pDGAT2-c19425(Ta), pYES-AtDGAT1, or pYES-BnDGAT1 can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Activity as indicated by the formation of TAG (as indicated, the mutant H1246is unable to synthesize TAG) can be measured either by relying on yeast-endogenous substrate-DAG, or by providing substrate in an *in vitro* assay.

For the former type of assay, cells are harvested after reaching stationary phase during in-

cubation at 28°C by centrifugation at 3000 x g for 5 min and resuspendet in 2 ml resuspention buffer (phosphate buffered saline (PBS) pH 7.4, see Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989). The equivalent of 200 mg cell pellet is taken, the volume adjusted to 210µl using PBS and 790 µl of methanol:chloroform (2:1) are added. Cells are disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm and lipids are extracted according to Blight and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917).

The *in vitro* assay is the preferred way of testing for DGAT activity, when activity is known or expected to be week when relying on endogenous substrate. Instead, both the type and concentration of the DAG acceptor molecule, as well as the type and concentration of the fatty acid-CoA can be controlled. To do so, cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspendet in 1 ml resuspention buffer (25 mM Tris/HCL pH 7.6) and disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant is transferred to a fresh tube and centrifuged at 3000 x g for 5 min. The obtained supernatant is the crude extract. Protein content is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 10 µl 50 nM [^{14}C]-6:0-DAG (giving about 3000 dpm/nmol), 50 µg of microsomal protein (the amount can be adjusted to stay within linear conditions without substrate limitation), 10 µl of 50 nM 18:3n-3-CoA or 50 nM 22:6n-3-CoA in assay buffer (50 mM Hepes buffer pH 7.2, 1 mg/ml BSA) to give a total volume of 100 µl. Samples are incubated for 10 min at 30°C.

In either case – in vivo or in vitro assay – lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using hexane:diethylether:acetic acid (70:30:1), and stained in iodine vapor. It can be seen by the formation of triacylglycerole (TAG) using the in vitro assay-conditions in figure 8, that pDGAT2-c19425mod(Ta) (SEQ-ID 52, lane 1 and 2), pDGAT2_c4648(No) (SEQ-ID 34, lane 5 and 6), pDGAT2_c48271(No) (SEQ-ID 102, lane 7 and 8), BnDGAT1 (SEQ-ID 107, lane 9 and 10), AtDGAT1 (SEQ-ID 105, lane 11 and 12), pDGAT2_c699(No) (SEQ-ID 19, lane 13 and 14) and pDGAT2_c2959(No) (SEQ-ID 25, lane 15) encode polypetides having DGAT activity.

Table 6 summarizes the results of the LPCAT, LPAAT and DGAT activity tests.

Table 6: Measured with microsomal protein and [14C]-18:1-LPA, [14C]-18:1-LPC or [14C]-6:0-1,2-DAG. Ofr the in vitro DGAT assay, 1 mg/ml of BSA was added to reduce activity for staying in the linear range.

Enzyme Class	Candidate	SEQ-IDs (ORF / protein / mRNA)	Activity in *vitro* using 18:3-CoA nmol/(mg*min)	Activity in *vitro* using 22:6-CoA nmol/(mg*min	Activity *in vivo*
LPAAT	pLPAAT_c6316(No)	13/14/15	81	64	

LPCAT	pLPAAT_c6316(No)	13/14/15	38	9	
DGAT	pDGAT2_c699(No)	19/20/21	0,22	0,17	Yes
DGAT	pDGAT2_c2959(No)	25/26/27	0,95	0,67	Yes
DGAT	pDGAT2_c4648(No)	34/35/36	1,4	0,17	Yes
DGAT	pDGAT2_c48271(No)	102/103/104	1,6	0	Yes
DGAT	pDGAT2-c19425(Ta)	52/53/54	4,0	5,6	Yes
DGAT	AtDGAT1	105/106/ --	1,6	1,2	Yes
DGAT	BnDGAT1	107/108/--	2,4	1,5	Yes

Example 8: Determination of substrate specificity for LPAAT

For determination of substrate specificities of the LPAAT enzymes, clones of the yeast mutant Y13749 (described in example 7) harboring LPAAT genes in the pYES plasmid can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 1-5 µg of microsomal protein (the amount is adjusted to achieve linear conditions without substrate limitation), 10 µl of 1 mM [^{14}C]-18:1-LPA (5000 dpm/nmol), 10 µl of 1 mM acyl-CoA in assay buffer (0.1 M phosphate buffer pH 7.2., 10 mg/ml Bovine Serum Albumine (BSA)) to give a total volume of 100 µl. Like to amount of microsomal protein added to the assay, also the amount of BSA has influence on observed anzmye activities, where higher amounts of BSA result on lower activities and lower amounts of BSA result in higher activities. The enzyme specificity can be tested for different acyl-CoA:s, e.g. 14:0-CoA, 16:0-CoA, 18:1-CoA, 18:2-CoA, 18:3-CoA, γ18:3-CoA, 18:4-CoA, 20:3-CoA, 20:4-CoA, 20:4(n-3)-CoA, 20:5-CoA, 22:5-CoA, 22:6-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The amount of phosphatidic acid (PA) produced in the reaction (and hence the enzyme activity) can be determined from the picture.

Example 9: Determination of substrate specificity for LPLAT

For LPCAT and LPEAT activity assay, clones of the yeast mutant Y12431 harboring LPLAT genes in the pYES plasmid can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1 Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain either 10µl 1 mM [^{14}C]-18:1-Lysophosphatidlycholine (-LPC), 5000 dpm/nmol (LPCAT assay) or 10µl 1 mM [^{14}C]-18:1-Lysophosphatidylethanolamine (-LPE), 5000 dpm/nmol (LPEAT assay), 1-10 µg of microsomal protein (the amount is adjusted to achieve linear conditions without substrate limitation), 10 µl of 1 mM acyl-CoA in assay buffer (0.1 M phosphate buffer pH 7.2., 10 mg/ml BSA) to give a total volume of 100 µl. Like to amount of microsomal protein added to the assay, also the amount of BSA has influence on observed anzmye activities, where higher amounts of BSA result on lower activities and lower amounts of BSA result in higher activities. The enzyme specificity can be tested for different acyl-CoA:s, e.g. 14:0-CoA, 16:0-CoA, 18:1-CoA, 18:2-CoA, 18:3-CoA, γ18:3-CoA, 18:4-CoA, 20:3-CoA, 20:4-CoA, 20:4(n-3)-CoA, 20:5-CoA, 22:5-CoA, 22:6-CoA.. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The amount of phosphatidyl choline (PC) or phosphatidyl ethanol amine (PE) produced in the reaction (and hence the enzyme activity) can be determined from the picture.

Example 10: Determination of substrate specificity for DGAT

For DGAT activity assay, clones of the yeast mutant H1246 harboring either one of pYES-pDGAT2_c699(No), pYES-pDGAT2_c2959(No), pYES-pDGAT2_c4648(No), pYES-pDGAT2_c48271(No), pYES-pDGAT2-c19425(Ta), pYES-AtDGAT1, or pYES-BnDGAT1 can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be in-

duced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 5 µl 1 mM [^{14}C]-6:0-DAG, 3000 dpm/nmol, 1-100 µg of microsomal protein (the amount is adjusted to achieve linear conditions without substrate limitation), 5 µl of 1 mM acyl-CoA in assay buffer (50 mM Hepes buffer pH 7.2, 1 mg/ml BSA) to give a total volume of 100 µl. The enzyme specificity can be tested for different acyl-CoA:s, e.g. 14:0-CoA, 16:0-CoA, 18:1-CoA, 18:2-CoA, 18:3-CoA, γ18:3-CoA, 18:4-CoA, 20:3-CoA, 20:4-CoA, 20:4(n-3)-CoA, 20:5-CoA, 22:5-CoA, 22:6-CoA.. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917).Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using hexane:diethylether:acetic acid (70:30:1), and autoradiographic pictures are taken using an instant imager (Packard). The amount of triacylglycerol (TAG) produced in the reaction (and hence the enzyme activity) can be determined from the picture. In Brassica napus and Arabidopsis, the DGAT involved in TAG-formation in seeds are of the DGAT1 type. The enzyme activity AtDGAT1 and BnDGAT1 for the different substrates can be seen in figure 9. The enzyme activity of pDGAT2-c19425(Ta) for the different substrates, compared to AtDGAT1 and BnDGAT1 is shown in figure 10. The enzyme activity of pDGAT2_c699(No) and pDGAT2_c4648(No) for the different substrates, compared to AtDGAT1 and BnDGAT1 is shown in figure 11. The data in figure 10 and 11 show clearly, that all DGAT2 enzymes shown in these figures vary strongly towards their activities for the various substrates, whereas the DGAT1 involved in TAG formation in Arabidopsis and Brassica napus exhibit less variability towards these different substrates.

Example 11: Determination of substrate selectivity for LPAAT

For determination of substrate selectivities of the LPAAT enzymes, clones of the yeast mutant Y13749 (described in example 7) harboring LPAAT genes can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption

buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. The substrate selectivity can be determined by mixing equimolar amounts of different acyl-CoA:s in the same reaction and measure the preference for using the different acyl groups as substrates. The assay is run as in the specificity studies (Example 5) but scaled up 18 times to get sufficient amount of PA for detection. Up to 4 different acyl-CoA:s can be used in the assay in equimolar amount instead of one single acyl-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The phosphatidic acid (PA) is recovered from the plate and the fatty acids methylated in situ on the gel with sulphuric acid (2%) in methanol. Fatty acid methyl esters are extracted with hexane and separated by gas-liquid chromatography (GLC) using a WCOT fused silica 50 m×0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard. The selectivity can be determined by calculating the amount of each acyl group that has been acylated to LPA.

Example 12: Determination of substrate selectivity for LPLAT

For LPCAT and LPEAT activity assay, clones of the yeast mutant Y12431 harboring LPLAt genes can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1 Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. The substrate selectivity can be determined

by mixing equimolar amounts of different acyl-CoA:s in the same reaction and measure the preference for using the different acyl groups as substrates. The assay is run as in the specificity studies (Example 6) but scaled up 18 times to get sufficient amount of PC or PE for detection. Up to 4 different acyl-CoA:s can be used in the assay in equimolar amount instead of one single acyl-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The PC or PE is recovered from the plate and the fatty acids methylated in situ on the gel with sulphuric acid (2%) in methanol. Fatty acid methyl esters are extracted with hexane and separated by gas-liquid chromatography (GLC) using a WCOT fused silica 50 m×0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard. The selectivity can be determined by calculating the amount of each acyl group that has been acylated to LPC or LPE.

Example 13: Determination of substrate selectivity for DGAT

For DGAT activity assay, clones of the yeast mutant H1246 harboring DGAT genes can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD_{600}=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. The substrate selectivity can be determined by mixing equimolar amounts of different acyl-CoA:s in the same reaction and measure the preference for using the different acyl groups as substrates. The assay is run as in the specificity studies (Example 7) but scaled up 18 times to get sufficient amount of TAG for detection. Up to 4 different acyl-CoA:s can be used in the assay in equimolar amount instead of one single acyl-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911–917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using

an instant imager (Packard). The TAG is recovered from the plate and the fatty acids methylated in situ on the gel with sulphuric acid (2%) in methanol. Fatty acid methyl esters are extracted with hexane and separated by gas-liquid chromatography (GLC) using a WCOT fused silica 50 m×0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard. The selectivity can be determined by calculating the amount of each acyl group that has been acylated to TAG.

Claims

1. A polynucleotide comprising a nucleic acid sequence elected from the group consisting of:
   a) a nucleic acid sequence having a nucleotide sequence as shown in any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55;
   b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in any one SEQ ID NOs: 53, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, and 56;
   c) a nucleic acid sequence being at least 40% identical to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having acyltransferase activity;
   d) a nucleic acid sequence encoding a polypeptide having acyltransferase activity and having an amino acid sequence which is at least 45% identical to the amino acid sequence of b); and
   e) a nucleic acid sequence which is capable of hybridizing under one of the following sets of conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having acyltransferase activity:
   f) hybridization in 50 mM Tris, pH 7.6, 6xSSC, 5xDenhardt's, 1.0% sodium dodecyl sulfat (SDS) 100µg denaturated calf thymus DNA at 34°C overnight and wash twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, repeat twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;
   g) hybridization in 6xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 0.5% SDS 100µg denaturated calf thymus DNA at 34°C overnight and wash twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, repeat twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;
   h) hybridization in 20-30% formamide, 5xSSPE, 5xDenhardt's solution, 1% SDS 100µg denaturated salmon sperm DNA at 34°C overnight and wash twice with 2xSSPE, 0.2%SDS at 42°C for 15 min each, repeat twice with 2xSSPE, 0.2%SDS at 55°C for 30 min each and repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;
   i) hybridization in 7% SDS, 0.5 M NaPO4, 1 mM EDTA at 50°C overnight and wash in 2 X SSC, 0.1% SDS at 50°C or 65°C;
   j) hybridization in 7% SDS, 0.5 M NaPO4, 1 mM EDTA at 50°C overnight and wash in 1 X SSC, 0.1% SDS at 50°C or 65°C; or
   k) hybridization in 7% SDS, 0.5 M NaPO4, 1 mM EDTA at 50°C overnight and wash in 0,1 X SSC, 0.1% SDS at 50°C or 65°C

2. The polynucleotide of claim 1, wherein said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

3. The polynucleotide of claim 1 or 2, wherein said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.

4. A vector comprising the polynucleotide of any one of claims 1 to 3.

5. A host cell comprising the polynucleotide of any one of claims 1 to 3 or the vector of claim 4.

6. A method for the manufacture of a polypeptide encoded by a polynucleotide of any one of claims 1 to 3 comprising
   a) cultivating the host cell of claim 5 under conditions which allow for the production of the said polypeptide; and
   b) obtaining the polypeptide from the host cell of step a).

7. A polypeptide encoded by the polynucleotide of any one of claims 1 to 3 or which is obtainable by the method of claim 6.

8. A non-human transgenic organism comprising the polynucleotide of any one of claims 1 to 3 or the vector of claim 4

9. The non-human transgenic organism of claim 8, which is a microorganism, a plant, plant part, or plant seed.

10. A method for the manufacture of polyunsaturated fatty acids comprising:
   a) cultivating the host cell of claim 5 under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
   b) obtaining said polyunsaturated fatty acids from the said host cell.

11. A method for the manufacture of polyunsaturated fatty acids comprising:
   a) cultivating the non-human transgenic organism of claim 8 or 9 under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
   b) obtaining said polyunsaturated fatty acids from the said non-human transgenic organism.

12. The method of claim 10 or 11, wherein said polyunsaturated fatty acid is arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

13. A method for the manufacture of an oil, lipid or fatty acid composition comprising the steps of the method of any one of claims 10 to 12 and the further step of formulating the polyunsaturated fatty acid as oil, lipid or fatty acid composition.

14. The method of claim 13, wherein said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or pharmaceuticals.

15. Oil, lipids or fatty acids or a fraction thereof produced by the method of any one of claims 10 to 12.

16. An oil, lipid or fatty acid composition comprising a polyunsaturated fatty acid obtainable by the method of any one of claims 10 to 12.

17. An antibody or a fragment derived thereof as an antigen which specifically recognizes a polypeptide encoded by the nucleic acid sequences of any one of the claims 1 to 3.

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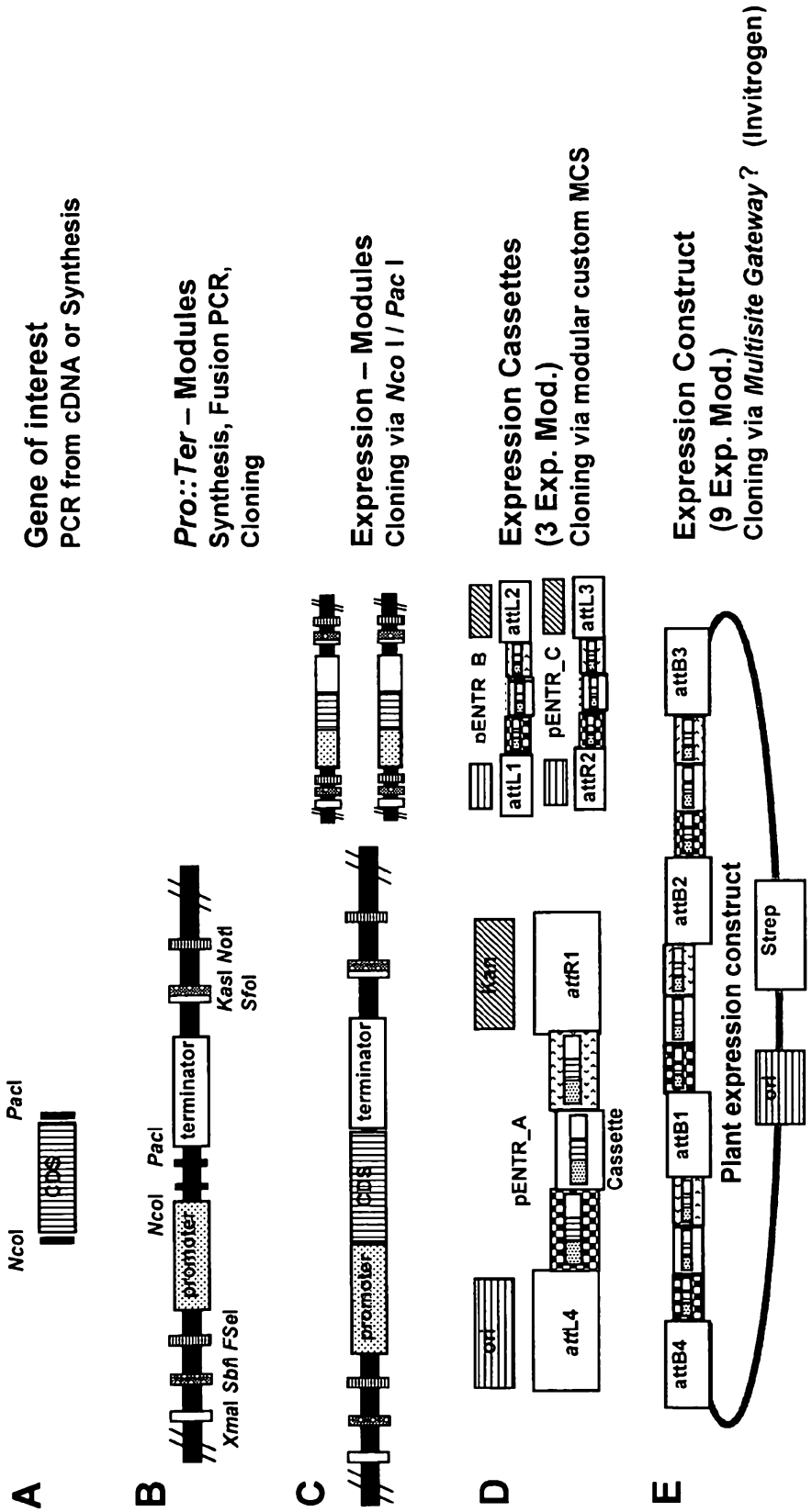
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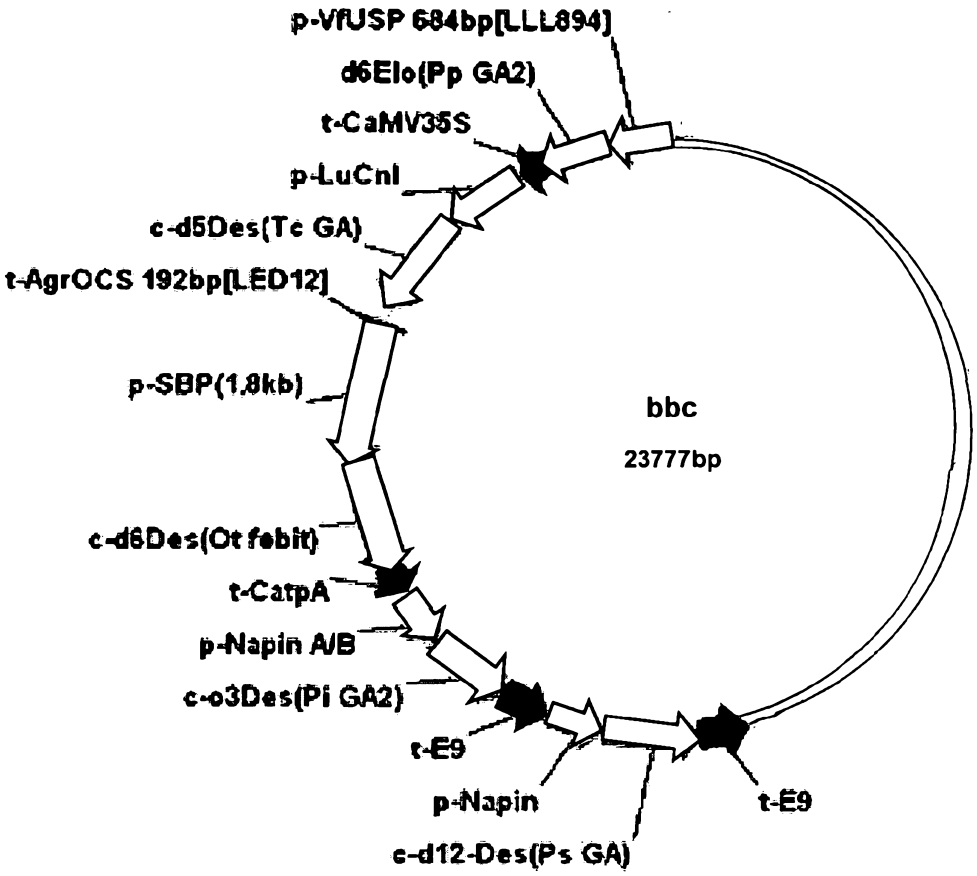
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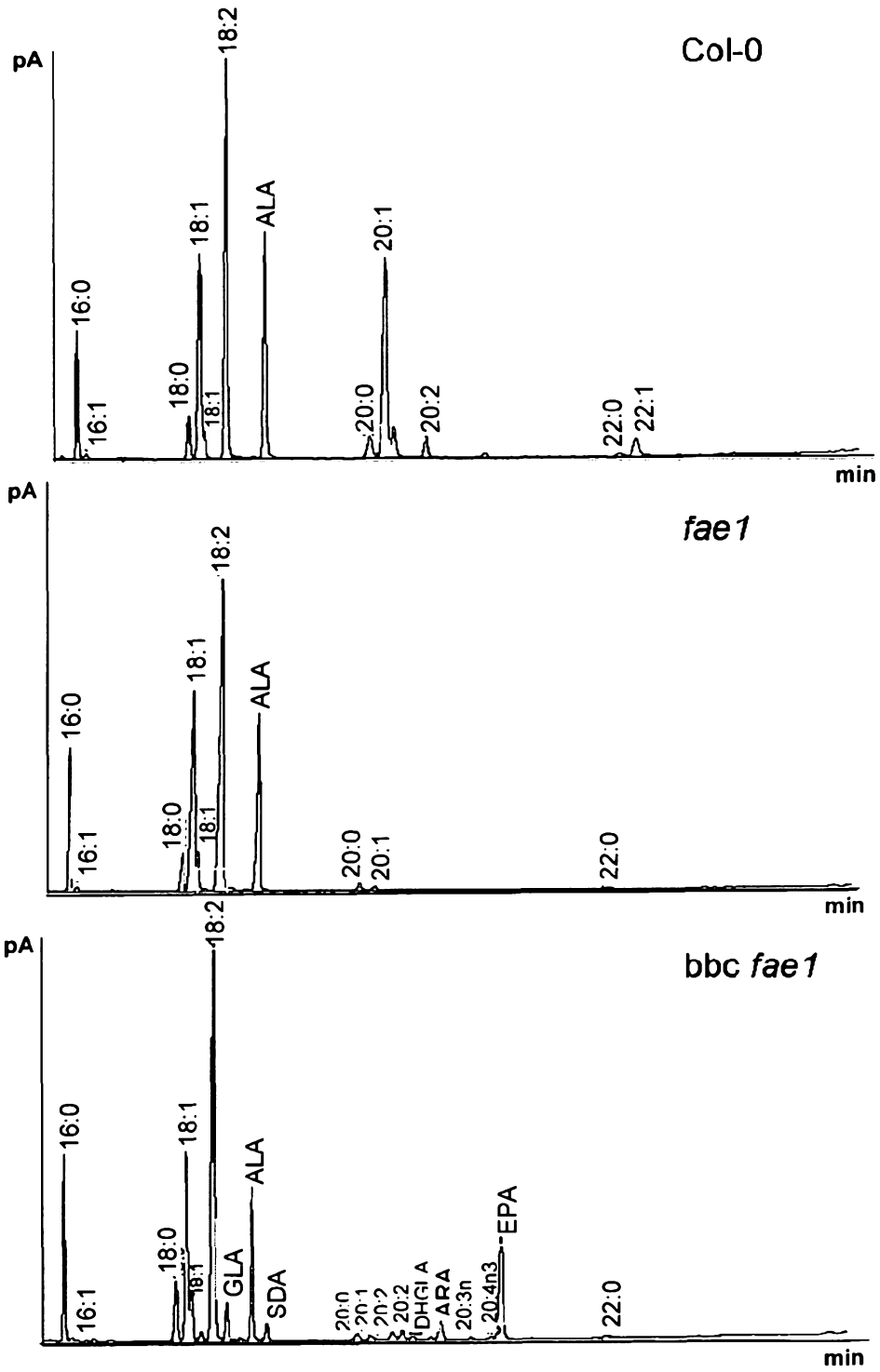
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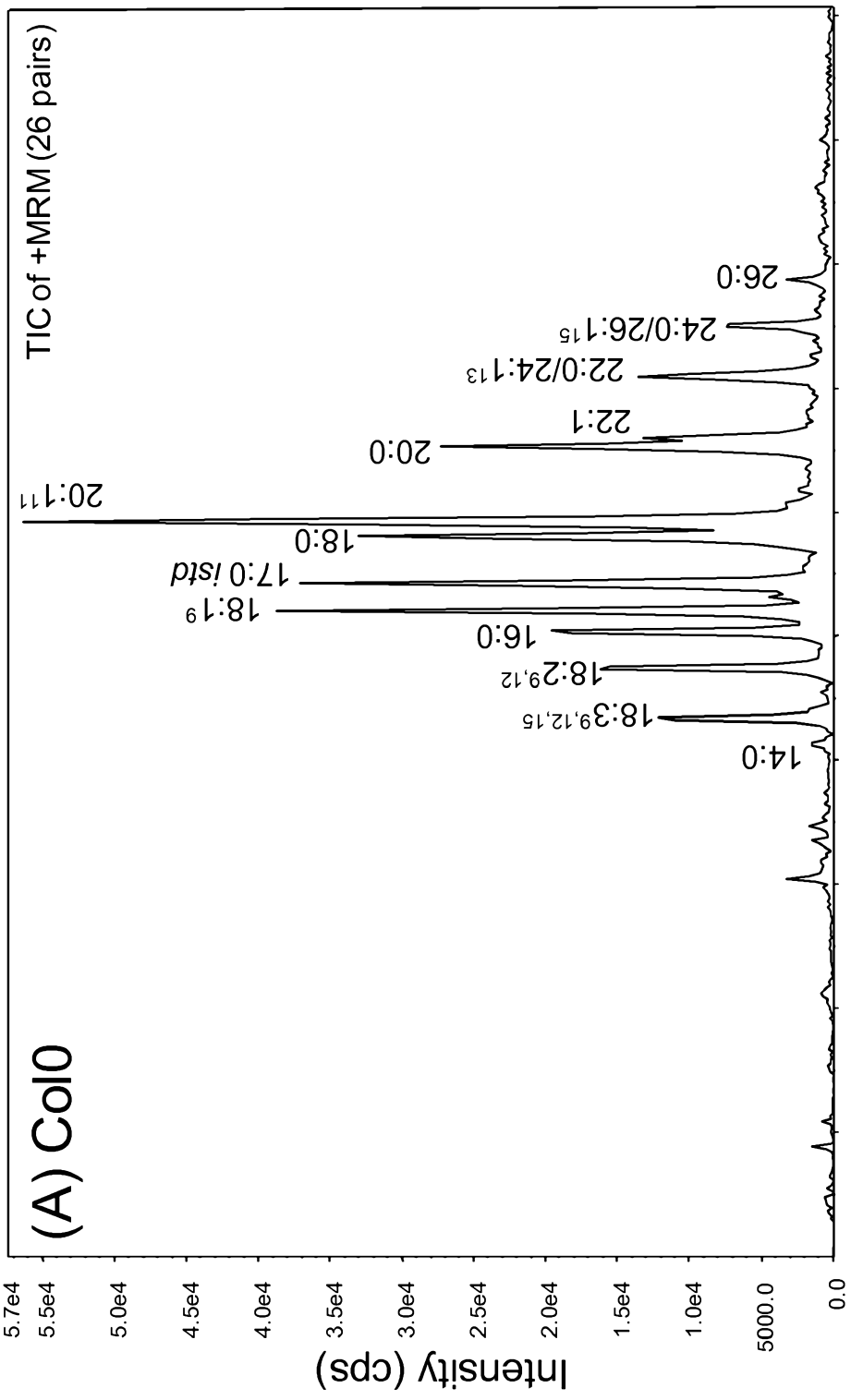
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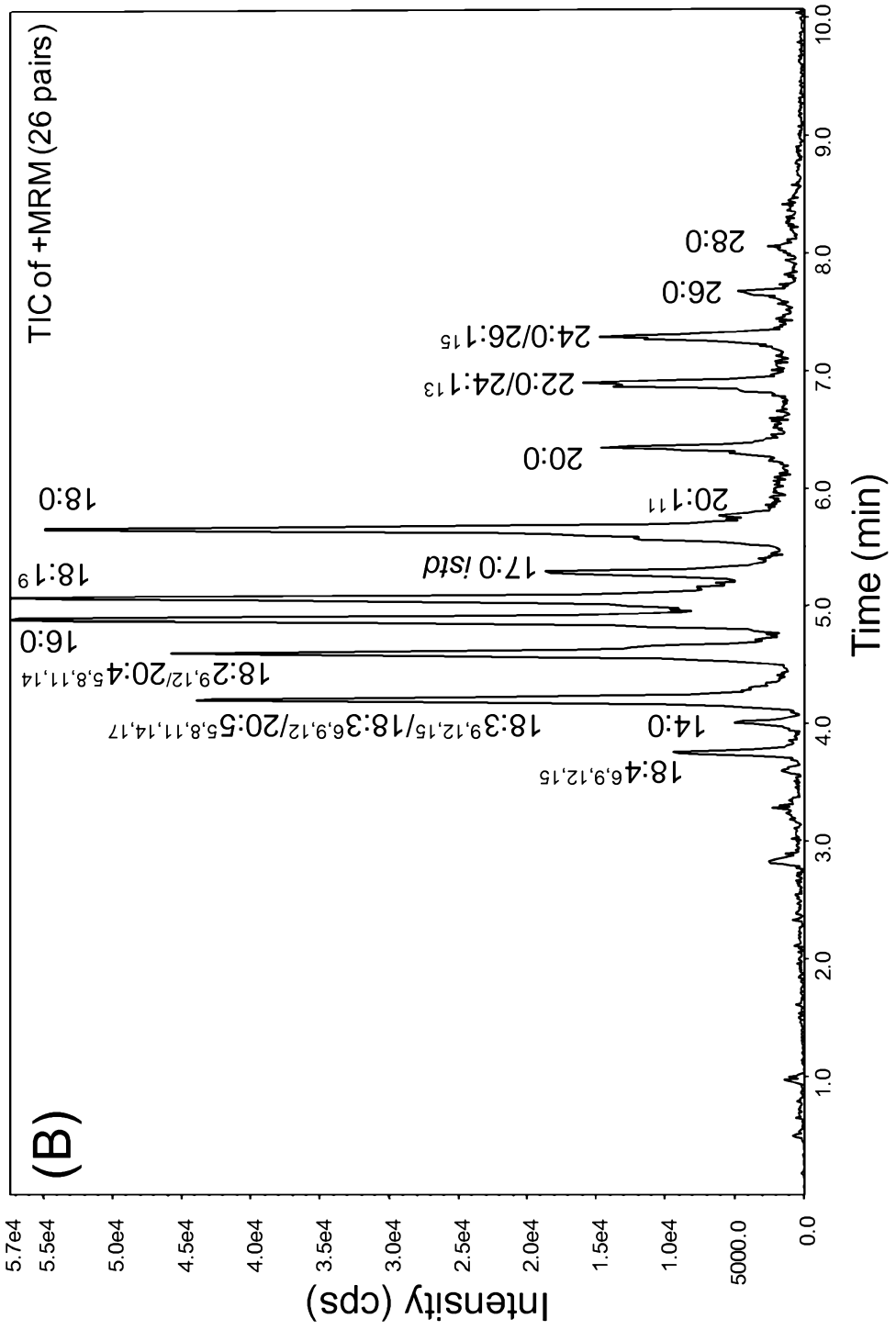

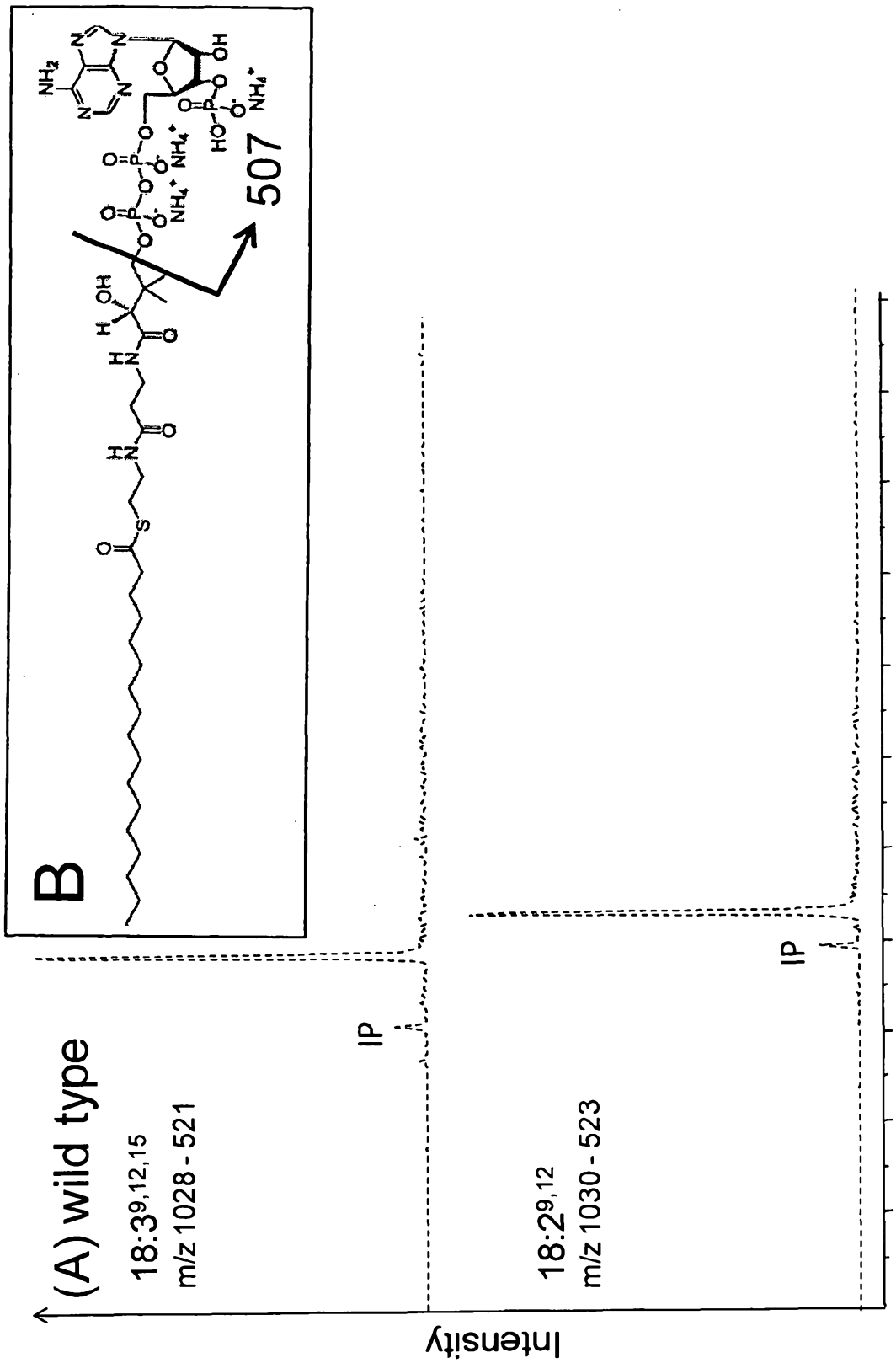
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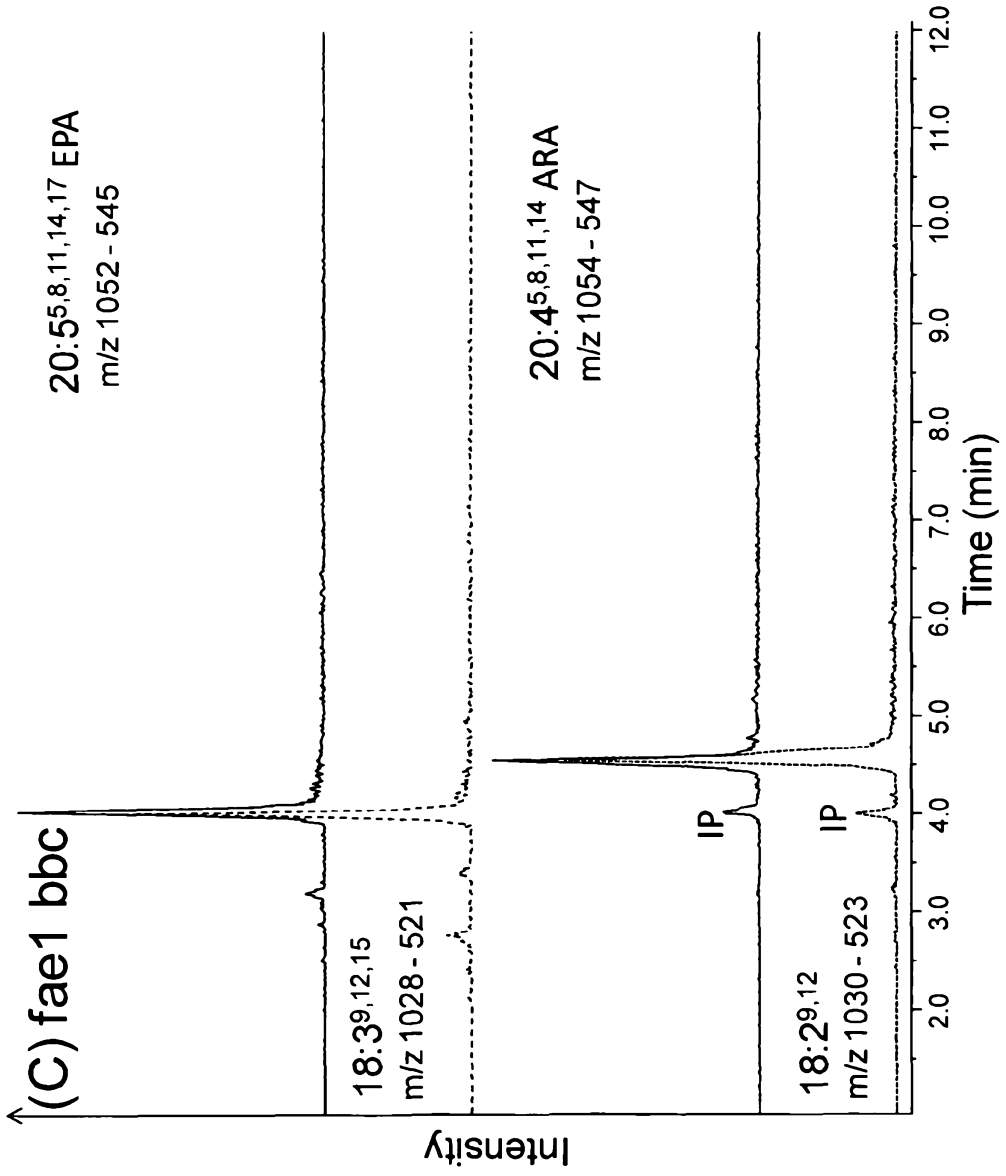

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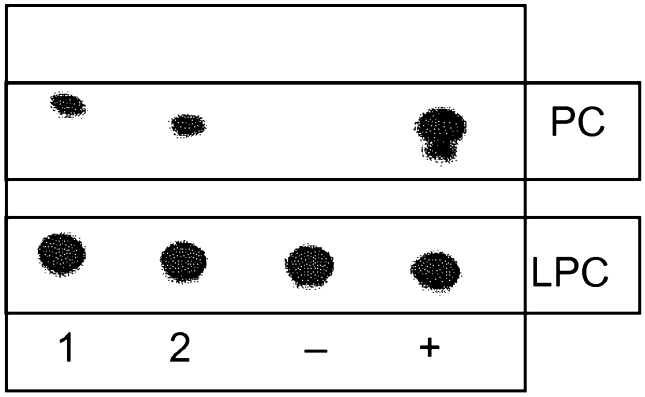

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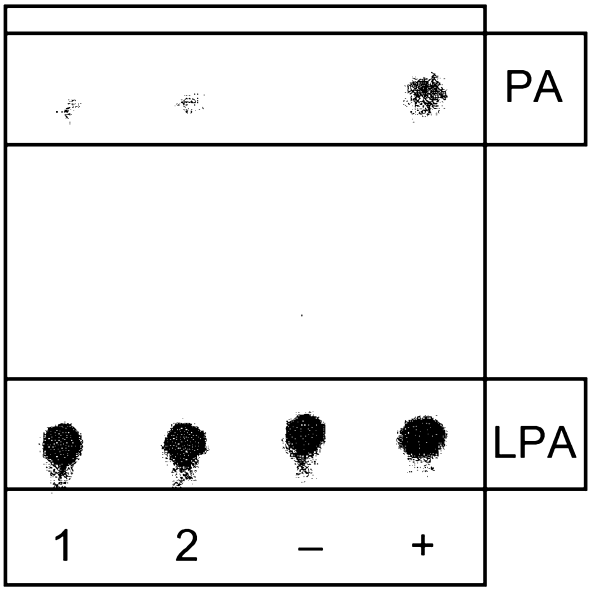

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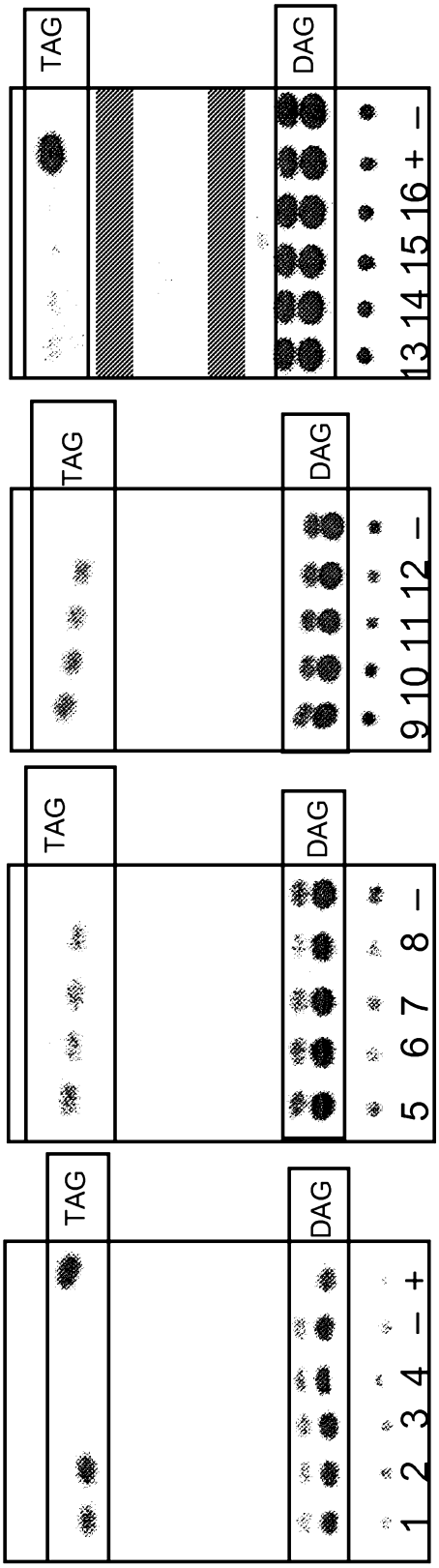

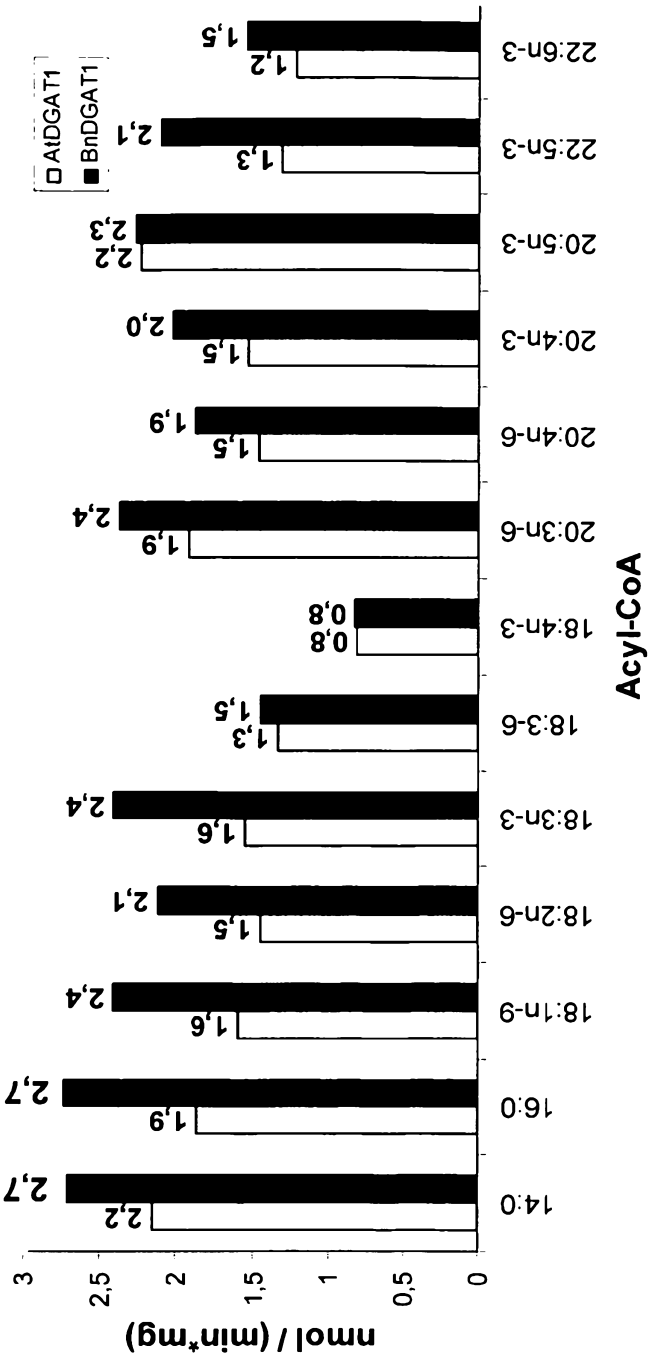
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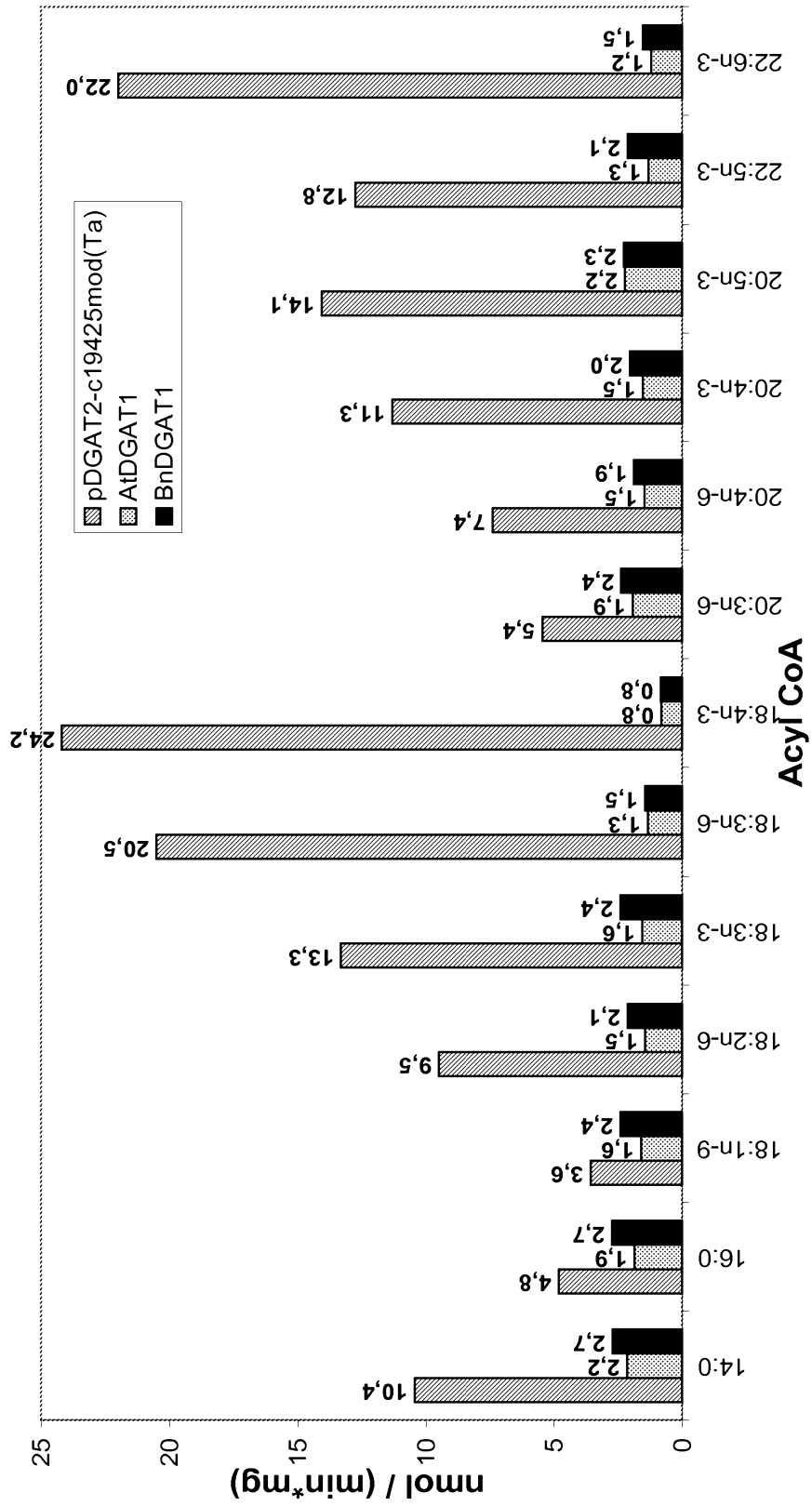
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Fig 10

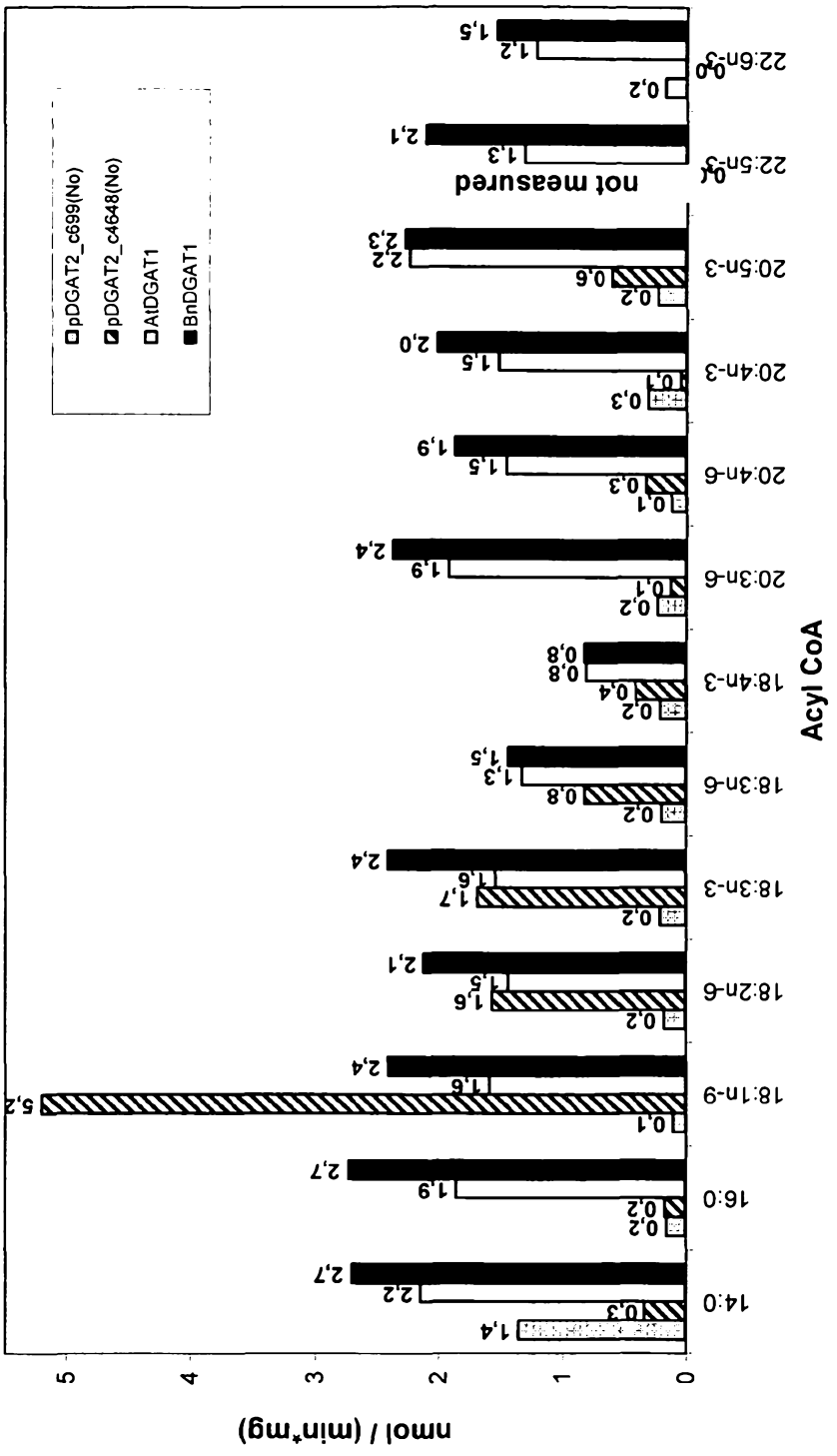
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Fig 11

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Gly Thr Gln Lys Trp Leu Gln Arg Tyr Val Tyr Phe Arg Asn Ser Glu
            340                 345                 350

Ser Leu Leu Ile Thr Tyr Phe Val Ser Ala Phe Trp His Gly Phe Tyr
        355                 360                 365

Pro Gly Tyr Tyr Leu Phe Phe Phe Ser Ile Ala Leu Val Gln Thr Val
    370                 375                 380

Gln Arg Ala Trp Gln Lys Lys Val Ser Pro Tyr Phe Thr Ser Thr Ile
385                 390                 395                 400

Pro Ala Leu Tyr His Leu Leu Cys Ile Leu Val Phe Ser Ala Tyr Ile
                405                 410                 415

Asn Tyr Phe Ser Ile Val Phe Gln Val Leu Ala Trp Asp Arg Ala Met
            420                 425                 430

Ala Val Trp Lys Ser Ala His Tyr Trp Gly His Val Ala Thr Ala Gly
        435                 440                 445

Ala Phe Val Leu Thr Ser Val Leu Pro Ser Pro Lys Lys Glu Ala Gly
    450                 455                 460

Lys Lys Val Leu Glu Glu Arg Lys Lys Ala Leu Arg Glu Gly Arg Arg
465                 470                 475                 480

Phe Arg Gly Lys Glu Glu Gly Leu Glu Gly Arg Lys Lys Val
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<210> 3
<211> 1908
<212> DNA
<213> Nannochloropsis oculata

<400> 3

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gaaggaggtt tagattcctc gcataaggca ttggaattta aatagtggtg ggcctgtctg 1800

gctttccgtg aaggagagca accataatgt gtgaccaacg ctttggcacc gcaaccacca 1860

taataacagc actaacaaaa agaagaacaa caataagaag gaggagat 1908

<210> 4

<211> 1776
<212> DNA
<213> Nannochloropsis oculata

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gagatgcttc aaactccctc ggcatcgtcg tcctcgccct cggcatcccc agagcgcaaa 300

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atgaacaata cttgggatat ctccaggttg gaacgcgtta aatgtgcgat attcggtcca 420

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aacgggacgt tgagcgctga tgagtttctg gcgttggcga ggtcaaatca acacttgtgc 1740

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<210> 5
<211> 591

<212> PRT
<213> Nannochloropsis oculata

<400> 5

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Tyr Thr Cys Ala Thr Ile Thr Ile Tyr Val Val Leu Gly Arg Phe Ser
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Ser Pro Asn Pro Arg Leu Arg Trp Leu Lys Leu Lys Asp Leu Glu Asn
        35                  40                  45

Ile Glu Thr Ala Asn Pro Ala Ala His Pro Ser Glu Ser Asp Ser Met
    50                  55                  60

Pro Leu Asn Ser Gly Asn Leu Ser Ser Ser Lys Pro Ile Ala Ala Ala
65                  70                  75                  80

Glu Met Leu Gln Thr Pro Ser Ala Ser Ser Ser Ser Pro Ser Ala Ser
                85                  90                  95

Pro Glu Arg Lys Ala Pro Met Met Arg Lys Leu Ser Phe Leu Ala Thr
            100                 105                 110

Thr Gly Val Ile Glu Asn Pro Phe Met Asn Asn Thr Trp Asp Ile Ser
        115                 120                 125

Arg Leu Glu Arg Val Lys Cys Ala Ile Phe Gly Pro Met Leu Ile Pro
    130                 135                 140

Pro Arg Leu Leu Leu Leu Phe Val Ser Leu Leu Gly Ala Tyr Gly Phe
145                 150                 155                 160

Gly Lys Leu Ser Thr Ile Gly Ala Glu Leu Glu Arg Pro Leu Pro Arg
                165                 170                 175

Trp Arg Ile Asp Leu Gln His Pro Met Lys Phe Phe Ala Arg Gly Ile
            180                 185                 190

Met Phe Ala Leu Gly Tyr His Trp Ile Ser Ile Lys Gly Lys Gln Ala
        195                 200                 205

Ser Pro Gln His Ala Pro Ile Val Val Ser Asn His Cys Ser Phe Cys
    210                 215                 220

Glu Ala Ile Tyr Leu Pro Gly Arg Leu Leu Ser Met Ala Val Ser Arg
225                 230                 235                 240

Arg Glu Asn Ala Ala Ile Pro Phe Phe Gly Gly Leu Met Gln Gln Val
                245                 250                 255
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Gln Cys Ile Phe Val Ser Arg Thr Asp Lys Asp Ser Arg Thr Thr Val
            260                 265                 270

Ala Asn Glu Ile Leu Arg Arg Ser Lys Ile Glu Arg Gly Gln Trp His
        275                 280                 285

Arg Gln Leu Leu Val Phe Pro Glu Gly Thr Thr Thr Asn Gly Ser Ala
    290                 295                 300

Val Ile Ser Phe Lys Val Gly Ser Phe Ala Gly Gly Val Ser Val Gln
305                 310                 315                 320

Pro Val Ala Val Ser Tyr Pro Ser Asn Gln Ile Cys Asp Pro Ser Trp
                325                 330                 335

Val Ser Gly Gly Pro His Pro Gly Glu Ile Leu Phe Lys Leu Leu Cys
            340                 345                 350

Gln Pro Trp Asn Ser Met Asn Val Thr Phe Leu Pro Val Tyr Asn Pro
        355                 360                 365

Asp Ala Ala Glu Ile Asp Asp Pro Val Leu Phe Ser Thr Asn Val Arg
    370                 375                 380

Arg Leu Ile Ala Ala Glu Leu Gly Val Pro Ala Ser Asp His Thr Phe
385                 390                 395                 400

Asp Asp Val Leu Leu Leu Met Glu Ala Lys Lys Leu Gly Tyr Gln Gly
                405                 410                 415

Gly Leu Arg Asp Cys Ile Ser Glu Leu Lys Asn Met Arg Lys Ile Leu
            420                 425                 430

Glu Ile Asp Leu Ala Lys Ala Lys Glu Tyr Leu His Glu Phe Ser Gln
        435                 440                 445

Leu Asp Thr Asn Arg Lys Gly Leu Leu Ser Tyr Pro Gln Phe Ile Lys
    450                 455                 460

Ala Phe Gly Ser Gln Asp Ser Asp Ala Leu Arg Ser Leu Phe Cys Val
465                 470                 475                 480

Leu Asp Val Gln Asp Arg Gly Val Ile Asn Leu Val Glu Tyr Thr Thr
                485                 490                 495

Gly Leu Ala Leu Leu Asn Glu Gln Gly Thr Asp Gly Phe Asp Gly Ala
            500                 505                 510

Met Arg Leu Ile Phe Lys Val Gln Asp Ser Ser Gly Glu Gly Arg Leu
        515                 520                 525
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Ser Lys Glu Asp Thr Ala Lys Val Leu Arg Arg Leu Trp Pro Asp Val
530 535 540

Thr Thr Glu Leu Phe Asp Ser Thr Phe Ala Ala Ala Asp Thr Asp Asn
545 550 555 560

Asn Gly Thr Leu Ser Ala Asp Glu Phe Leu Ala Leu Ala Arg Ser Asn
565 570 575

Gln His Leu Cys Pro Ser Leu Lys Ser Ser Leu Cys Gly Arg Leu
580 585 590

<210> 6
<211> 2247
<212> DNA
<213> Nannochloropsis oculata

<400> 6
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atcacaacta tgaccacgac tgtcatctct agctcgatgg ggcccatcct ggcctattat 180

acgtgtgcca caatcaccat ctacgtagtg ctcggccgct tttccagtcc aaacccgcgc 240

ttgagatggc tgaagctcaa agacctggag aacattgaga ctgcgaaccc ggccgcgcac 300

ccttcagagt ctgattctat gcctcttaat tctggcaatc tatcgtcttc caagcccatt 360

gccgcagctg agatgcttca aactccctcg gcatcgtcgt cctcgccctc ggcatcccca 420

gagcgcaaag ctcctatgat gcggaagctt tcctttctcg ccacgactgg agtcatcgaa 480

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cgcatcgacc tgcagcaccc catgaagttt tttgcccgcg ggattatgtt tgcattgggc 720

taccattgga tctccatcaa aggaaagcaa gcaagcccgc aacacgctcc tatcgttgtc 780

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<210> 7
<211> 1134
<212> DNA
<213> Thraustochytrium aureum

<400> 7
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<210> 8
<211> 377
<212> PRT
<213> Thraustochytrium aureum

<400> 8

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Gln Ser Arg Lys Pro Arg Pro Trp Asn Trp Asn Ala Tyr Leu Trp Pro
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Leu Trp Ala Ala Gly Val Phe Ile Arg Tyr Phe Val Leu Phe Pro Ile
35 40 45

Arg Leu Ala Ile Phe Ala Met Gly Trp Ile Leu Phe Gly Ile Gly Met
50 55 60

Leu Val Thr Gln Thr Cys Phe Pro His Gly Pro Arg Arg Thr Ser Leu
65 70 75 80

Glu His Gly Leu Ile Ser Met Met Cys Gly Val Phe Cys Ile Thr Trp
85 90 95

Gly Ala Val Ile Arg Tyr His Gly Ser Pro Val Lys Pro Arg Glu Gly
100 105 110

Glu Cys Gln Pro Val Tyr Val Ala Asn His Thr Ser Met Ile Asp Val
115 120 125

Ile Ile Leu Gln Gln Met Arg Cys Phe Ser Leu Val Gly Gln Arg His
130 135 140

Lys Gly Ile Val Arg Phe Leu Gln Glu Val Val Leu Gly Cys Leu Gln
145 150 155 160

Cys Val Trp Phe Asp Arg Gly Glu Ile Lys Asp Arg Ala Ala Val Ala
165 170 175

Arg Lys Leu Asn Glu His Ala Asn Asp Pro Thr Arg Asn Pro Leu Leu
180 185 190

Val Phe Pro Glu Gly Thr Cys Val Asn Asn Glu Tyr Val Ile Gln Phe
195 200 205

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Lys Lys Gly Ile Phe Glu Ile Gly Ala Pro Val Val Pro Val Ala Ile
    210                 215                 220

Lys Tyr Asn Lys Met Phe Val Asp Pro Phe Trp Asn Ser Arg Ala Gln
225                 230                 235                 240

Ser Phe Pro Met His Leu Val Glu Leu Met Thr Ser Trp Cys Leu Ile
                245                 250                 255

Cys Glu Val Trp Tyr Leu Lys Pro Leu Glu Arg Met Glu Arg Glu Ser
            260                 265                 270

Ser Thr Asp Phe Ala Ala Arg Val Lys Lys Ala Ile Ala Asp Gln Ala
        275                 280                 285

Gly Leu Lys Asn Val Asn Trp Asp Gly Tyr Met Lys Tyr Trp Lys Pro
    290                 295                 300

Ser Glu Arg Tyr Leu Arg Ala Arg Gln Ala Ile Phe Ala Lys Thr Leu
305                 310                 315                 320

Arg Lys Ile His Ser Arg Ser Leu Glu Gln Asp Lys Ala Asp Arg Gln
                325                 330                 335

Ala Ile Leu His Asp Leu Asp Gly Ala Phe Pro Asp Ser Gly Thr His
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Arg Gly Glu Arg Glu Ser Pro Arg Glu Pro Gly Leu Arg Arg Arg Gln
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<210> 9
<211> 1288
<212> DNA
<213> Thraustochytrium aureum

<400> 9

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<210> 10
<211> 1284
<212> DNA
<213> Nannochloropsis oculata

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gcgcggcggc caccaggctt ccactcgata gggcgatcat cagccacgac taggagaata 180

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ggtgcattca aggtggcgac caaggcgggc gtgcctatag tgcctgtatc tatcgcgggg 1080

acgcatgtca tgatgcccaa ggaggtgatc atgcctcaat gtgctggccg gggaatcacc 1140

gccattcatg tccaccctcc catctccatc aagggccgca cggaccagga gctgtcggat 1200

ctggcgtttg atactattaa caatgcattg tcagatgagc agcgggctat gcctagcagg 1260

aagaaggacg attcgagagc ttaa 1284

<210> 11
<211> 427
<212> PRT
<213> Nannochloropsis oculata

<400> 11

Met Lys Thr Pro Thr Ser Leu Ala Cys Gly Ala Cys Thr Ala Ala Val
1 5 10 15

Leu Met Cys Phe Thr Thr Thr Ala Asp Ala Leu Ala Ser Thr Ser Gln
20 25 30

Pro Gly Ser Val Gly Val Ala Val Ala Arg Arg Pro Pro Gly Phe His
35 40 45

Ser Ile Gly Arg Ser Ser Ala Thr Thr Arg Arg Ile Ser Arg Gly Gly
50 55 60

Ile Glu Asp Leu Gly Thr His His Thr Trp Gly Gly Arg Met Ser Gln
65 70 75 80

Gln His Gln Gln His Gln Gln His Gln Gln His Arg Arg Arg Arg Arg
85 90 95

Thr Pro Thr Met Leu Val Glu Thr Asp Val Lys Val Lys Glu Glu Ala
100 105 110

Gly Ile Gly His Gly Ser Gly Ser Asn Glu Ser Gly Asn Arg Ser Gly
115 120 125

Lys Ser Gly Ser Ala Ala Ala Asp Ala Ser Glu Gly Thr Gly Pro Pro
130 135 140

Pro Val Pro Val Asp Thr Phe Arg His Lys Ser Leu Ala Glu Val Pro
145 150 155 160

Thr Asp Tyr Gly Pro Tyr Leu Thr Ile Lys Gly Phe Lys Ile Asn Ala
165 170 175

Phe Gly Phe Tyr Phe Cys Phe Val Ala Leu Phe Trp Ala Ile Pro Trp
180 185 190

Gly Val Phe Leu Ile Leu Tyr Lys Ala Ser Leu Glu Phe Met Asp Lys
195 200 205

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Ile Asp Pro Arg Arg Tyr Asn Val Asp Arg Ser Ser Ser Leu Trp Gly
    210                 215                 220

Trp Leu Thr Ser Ile Ser Thr Asp Ser Leu Pro Asp Ile Thr Gly Met
225                 230                 235                 240

Glu Asn Ile Pro Lys Gly Pro Ala Val Phe Val Ala Asn His Ala Ser
                245                 250                 255

Trp Met Asp Val Pro Tyr Thr Ala Gln Leu Pro Ile Arg Ala Lys Tyr
            260                 265                 270

Leu Ala Lys Ala Asp Leu Ala Lys Ile Pro Ile Leu Gly Asn Ala Met
        275                 280                 285

Ser Met Ala Gln His Val Leu Leu Asp Arg Asp Asp Lys Arg Ser Gln
    290                 295                 300

Met Glu Ala Leu Arg Ser Ala Leu Leu Ile Leu Lys Thr Gly Thr Pro
305                 310                 315                 320

Ile Phe Val Phe Pro Glu Gly Thr Arg Gly Pro Gln Gly Arg Met Gln
                325                 330                 335

Thr Phe Lys Met Gly Ala Phe Lys Val Ala Thr Lys Ala Gly Val Pro
            340                 345                 350

Ile Val Pro Val Ser Ile Ala Gly Thr His Val Met Met Pro Lys Glu
        355                 360                 365

Val Ile Met Pro Gln Cys Ala Gly Arg Gly Ile Thr Ala Ile His Val
    370                 375                 380

His Pro Pro Ile Ser Ile Lys Gly Arg Thr Asp Gln Glu Leu Ser Asp
385                 390                 395                 400

Leu Ala Phe Asp Thr Ile Asn Asn Ala Leu Ser Asp Glu Gln Arg Ala
                405                 410                 415

Met Pro Ser Arg Lys Lys Asp Asp Ser Arg Ala
            420                 425


<210>  12
<211>  1826
<212>  DNA
<213>  Nannochloropsis oculata

<400>  12
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acgacaagtg attcatgaag acgcccacga gcctggcgtg cggagcctgc acggcagccg      120

tgttaatgtg tttcacaaca acagcagatg cccttgccag cacatcacaa ccgggcagcg      180
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ttggcgtggc tgtcgcgcgg cggccaccag gcttccactc gatagggcga tcatcagcca 240

cgactaggag aataagcagg ggagggatag aggatctcgg aacccatcac acgtggggcg 300

gcaggatgtc gcagcagcac cagcagcacc agcagcacca gcagcaccgt cggcgtagga 360

ggacacccac tatgctagtg gagacagacg tgaaggtaaa agaggaagcg gggattggcc 420

acggatcagg aagcaacgaa agtggcaaca ggagcggcaa gagcgggtct gcggcggcag 480

acgcctcaga aggtacaggc ccaccgccag tgcccgtgga taccttccgg cacaagagct 540

tggcggaggt cccgacggac tatggaccct acctgaccat taaagggttc aagatcaatg 600

cctttggctt ctatttctgc ttcgtggccc tattctgggc gatcccctgg ggtgtcttcc 660

tcatcctgta caaggcgagt ttggagttca tggacaagat cgatcctcgc cggtacaacg 720

tggaccgctc cagttcccta tggggctggc tgaccagtat cagtactgac tccttacccg 780

acattacggg catggagaac attcccaagg gaccggcggt cttcgtcgcc aaccacgcct 840

cctggatgga cgtgccctac actgcccaac tgcccatccg cgccaagtac ctagcgaaag 900

ctgacctggc caagatccca atcctgggca acgccatgag catggctcag cacgtcctcc 960

tcgatcgaga cgacaagcgc agtcaaatgg aagccctgcg ctctgctctc ctgatcctca 1020

agacaggcac ccccatcttc gtcttccccg agggcacccg tgggcctcaa ggccgaatgc 1080

agacctttaa gatgggtgca ttcaaggtgg cgaccaaggc gggcgtgcct atagtgcctg 1140

tatctatcgc ggggacgcat gtcatgatgc ccaaggaggt gatcatgcct caatgtgctg 1200

gccggggaat caccgccatt catgtccacc ctcccatctc catcaagggc cgcacggacc 1260

aggagctgtc ggatctggcg tttgatacta ttaacaatgc attgtcagat gagcagcggg 1320

ctatgcctag caggaagaag gacgattcga gagcttaaga agaaggaaaa gagaagatgt 1380

gaaggaatga ggtgaaggca tgtcaacaat aggagataga gatcatgaag agatgagagc 1440

gagggaatca aaacccgttc agtaagccct gtgtagatca tatgcaggaa aagtgagcaa 1500

caggagcggc aggagaagca gttgggcgca tcgagaaaga caattaccaa gcaggaggca 1560

ataaaaggca attatcgaat agatttggag cgggggggtca gcgcacagcc gaacaagatg 1620

ccgtgtgctt agcagcagca gaatccgacc atagcgtaaa cctcacgaat gtttgtggtg 1680

agaagatggc aaatcaaatt cttcatcgtt tgtttgcaat tggtgatgca tgagattcct 1740

atagaccaga gagactggga agcttcacct ggagtaacag aaagaaagac taacagacga 1800

caacaaaaaa aaaaaaaaaa aaaaaa 1826

<210> 13
<211> 1395
<212> DNA
<213> Nannochloropsis oculata

<400> 13
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agcaacggcg ccaacgtccc gtcgtccacc tcctctacag cctccgcttc ttcctcctcc 120

aaaggcaccc tacccgcacg tgtccaggcc ctgcaaacga aggccgccac attgcctcag 180

cctttatcga atgtggcaaa acgcgccttg tactacgagg cggaaatgct ctggcaatca 240

atcaaggatg agctgcccgc cgagcacccg gaccaggcct ctttacttgc ggcaatcgac 300

cagttcgaga ccaaccttct acgcatcagt cccgctcagc tcgccaccac ctctttacga 360

cggatcctac aacaactcga catgctcctg cgaatcatta cttgctccct ctacctctgc 420

cttctagggg tcatcacatt tttgcccatg atcactctcg ttcccatcct cgaccgcctc 480

ctcgtaatcc tgggctggcc ccgtcgtttc ctcatctacg aactggccaa aaaggcatct 540

gcacgtggat ttctctacct ggccggtgtt ttctacacgg aagaagggaa gcaagccaat 600

gggtatgaaa ccccccttgt cctcctcttt caacacggct cgaaccttga tggcttcttg 660

atcttggatt cctttcctca attctttaaa tcaatcggga aagacgacat ctttctcatg 720

ccttacgtag ggtggatggc atatgtgtac ggcattctac ctatcgaccg caagcatcgt 780

aacgaagcaa tcaaacagct aggacgagcc acccgcgtct gtacctctgg tgtggccgtc 840

gctctttccc ccgaggggac acgtagcaag accggacaat tgatgcgatt caagaaaggg 900

ccgttttact tacaagccga gacatcggct actgtcaccc ctcttgtcat cgttggaaat 960

tacgagttgt ggcctccaaa ctatttcttt acctgtcctg ggcaggtggt gatgaggtat 1020

ctccccccca ttgaccattc ctccctccct ccctcggttg gtcggaacaa agacgagttc 1080

agtcgatatg tgcgcaagca gatgtttgag gccattgatg atatcatggc tggttccgag 1140

gagggaggga aggaggtagg ggagaagagg aaaaaatatg cgccgggggg gaaattgacc 1200

tggtggttgc ggggagtgaa tttggcatgc atgtgcctgt tttggttgat ggtaaaggcg 1260

gcgtggatgg tggtaacggg ggtgagtgac gcgtatgggt tcagtagggg ggcgttggcg 1320

gggggattcg ttgcatacac ggtgagtgtg actgctggcc tgtatatatt gtactgcaag 1380

gcgccggcgt cgtga 1395

<210> 14
<211> 464
<212> PRT
<213> Nannochloropsis oculata

<400> 14

Met Val Arg Arg Lys Met Asp Val Asp Ser Ser Ala Ala Gly Glu Ala
1 5 10 15

Ala Ser Ala Thr Ser Asn Gly Ala Asn Val Pro Ser Ser Thr Ser Ser
20 25 30

Thr Ala Ser Ala Ser Ser Ser Ser Lys Gly Thr Leu Pro Ala Arg Val
35 40 45

Gln Ala Leu Gln Thr Lys Ala Ala Thr Leu Pro Gln Pro Leu Ser Asn
50 55 60

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Val Ala Lys Arg Ala Leu Tyr Tyr Glu Ala Glu Met Leu Trp Gln Ser
65                  70                  75                  80

Ile Lys Asp Glu Leu Pro Ala Glu His Pro Asp Gln Ala Ser Leu Leu
                85                  90                  95

Ala Ala Ile Asp Gln Phe Glu Thr Asn Leu Leu Arg Ile Ser Pro Ala
            100                 105                 110

Gln Leu Ala Thr Thr Ser Leu Arg Arg Ile Leu Gln Gln Leu Asp Met
        115                 120                 125

Leu Leu Arg Ile Ile Thr Cys Ser Leu Tyr Leu Cys Leu Leu Gly Val
    130                 135                 140

Ile Thr Phe Leu Pro Met Ile Thr Leu Val Pro Ile Leu Asp Arg Leu
145                 150                 155                 160

Leu Val Ile Leu Gly Trp Pro Arg Arg Phe Leu Ile Tyr Glu Leu Ala
                165                 170                 175

Lys Lys Ala Ser Ala Arg Gly Phe Leu Tyr Leu Ala Gly Val Phe Tyr
            180                 185                 190

Thr Glu Glu Gly Lys Gln Ala Asn Gly Tyr Glu Thr Pro Leu Val Leu
        195                 200                 205

Leu Phe Gln His Gly Ser Asn Leu Asp Gly Phe Leu Ile Leu Asp Ser
    210                 215                 220

Phe Pro Gln Phe Phe Lys Ser Ile Gly Lys Asp Asp Ile Phe Leu Met
225                 230                 235                 240

Pro Tyr Val Gly Trp Met Ala Tyr Val Tyr Gly Ile Leu Pro Ile Asp
                245                 250                 255

Arg Lys His Arg Asn Glu Ala Ile Lys Gln Leu Gly Arg Ala Thr Arg
            260                 265                 270

Val Cys Thr Ser Gly Val Ala Val Ala Leu Ser Pro Glu Gly Thr Arg
        275                 280                 285

Ser Lys Thr Gly Gln Leu Met Arg Phe Lys Lys Gly Pro Phe Tyr Leu
    290                 295                 300

Gln Ala Glu Thr Ser Ala Thr Val Thr Pro Leu Val Ile Val Gly Asn
305                 310                 315                 320

Tyr Glu Leu Trp Pro Pro Asn Tyr Phe Phe Thr Cys Pro Gly Gln Val
                325                 330                 335
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Val Met Arg Tyr Leu Pro Pro Ile Asp His Ser Ser Leu Pro Pro Ser
            340                 345                 350

Val Gly Arg Asn Lys Asp Glu Phe Ser Arg Tyr Val Arg Lys Gln Met
        355                 360                 365

Phe Glu Ala Ile Asp Asp Ile Met Ala Gly Ser Glu Glu Gly Gly Lys
    370                 375                 380

Glu Val Gly Glu Lys Arg Lys Lys Tyr Ala Pro Gly Gly Lys Leu Thr
385                 390                 395                 400

Trp Trp Leu Arg Gly Val Asn Leu Ala Cys Met Cys Leu Phe Trp Leu
                405                 410                 415

Met Val Lys Ala Ala Trp Met Val Val Thr Gly Val Ser Asp Ala Tyr
            420                 425                 430

Gly Phe Ser Arg Gly Ala Leu Ala Gly Gly Phe Val Ala Tyr Thr Val
        435                 440                 445

Ser Val Thr Ala Gly Leu Tyr Ile Leu Tyr Cys Lys Ala Pro Ala Ser
    450                 455                 460
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<210> 15
<211> 1771
<212> DNA
<213> Nannochloropsis oculata

<400> 15

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atttttcagc aaagtaatca agataataaa caaaaacaat cctataaagg aaaaacaaca       60

ggacaaatca atggtcagga ggaagatgga cgtggacagc tcggccgccg gcgaagcggc      120

gtcagctacg agcaacggcg ccaacgtccc gtcgtccacc tcctctacag cctccgcttc      180

ttcctcctcc aaaggcaccc tacccgcacg tgtccaggcc ctgcaaacga aggccgccac      240

attgcctcag cctttatcga atgtggcaaa acgcgccttg tactacgagg cggaaatgct      300

ctggcaatca atcaaggatg agctgcccgc cgagcacccg gaccaggcct ctttacttgc      360

ggcaatcgac cagttcgaga ccaaccttct acgcatcagt cccgctcagc tcgccaccac      420

ctctttacga cggatcctac aacaactcga catgctcctg cgaatcatta cttgctccct      480

ctacctctgc cttctagggg tcatcacatt tttgcccatg atcactctcg ttcccatcct      540

cgaccgcctc ctcgtaatcc tgggctggcc ccgtcgtttc ctcatctacg aactggccaa      600

aaaggcatct gcacgtggat ttctctacct ggccggtgtt ttctacacgg aagaagggaa      660

gcaagccaat gggtatgaaa ccccccttgt cctcctcttt caacacggct cgaaccttga      720

tggcttcttg atcttggatt cctttcctca attctttaaa tcaatcggga aagacgacat      780

ctttctcatg ccttacgtag ggtggatggc atatgtgtac ggcattctac ctatcgaccg      840

caagcatcgt aacgaagcaa tcaaacagct aggacgagcc acccgcgtct gtacctctgg      900
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tgtggccgtc gctctttccc ccgaggggac acgtagcaag accggacaat tgatgcgatt 960

caagaaaggg ccgttttact tacaagccga gacatcggct actgtcaccc ctcttgtcat 1020

cgttggaaat tacgagttgt ggcctccaaa ctatttcttt acctgtcctg ggcaggtggt 1080

gatgaggtat ctccccccca ttgaccattc ctccctccct ccctcggttg gtcggaacaa 1140

agacgagttc agtcgatatg tgcgcaagca gatgtttgag gccattgatg atatcatggc 1200

tggttccgag gagggaggga aggaggtagg ggagaagagg aaaaaatatg cgccgggggg 1260

gaaattgacc tggtggttgc ggggagtgaa tttggcatgc atgtgcctgt tttggttgat 1320

ggtaaaggcg gcgtggatgg tggtaacggg ggtgagtgac gcgtatgggt tcagtagggg 1380

ggcgttggcg gggggattcg ttgcatacac ggtgagtgtg actgctggcc tgtatatatt 1440

gtactgcaag gcgccggcgt cgtgagaggg gggaagggag gggggaagga gagatagaag 1500

acgaggtaga ggtagatgtg agtgtgagat agcgcgagta ttatcttgaa gaaaagagat 1560

gaattgtagt agaagagtcg ggtattttag cagggagaga atattgtatg gagggtaaac 1620

gtgtgggaaa gaggagggag ggacctgaga tggataatga aagaatacta gagagagcgc 1680

gtgacacgtt cattgcttcc tcggattagt tgcctgtgca taagttaaag ataatagaga 1740

ggaatggcgc tcgcatgctc ctctttacac t 1771

<210> 16
<211> 1026
<212> DNA
<213> Nannochloropsis oculata

<400> 16
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cccaagcgcc ccaatccgca gtactggcgg tatgcaagcc ttgccgcctt ccttctcact 120

tgcttcctgg ccccttccag taactcgtgg gccaccaccc tccgccgcgc ctgctgggcg 180

gcgtactgga cgacctacct ggacacaagc tataaggacg gctcacgggc ctggccctgg 240

tttcagcgat tgcgaatctg gcgtatgtat tgcggctatt tgcagggcaa agtcatttgc 300

acggtgccct tggacccggc gcagcaattt atcttcgcgg cccatcccca cggcattggt 360

acctggaacc atttcctgac catgactgac ggctgtcgat ttctctcctc ctcctacccc 420

cgcccgcggc tcgacctggg tgcgacagta cttttcttca tccccttctt aaaggaaatt 480

ctgctttggc taggctgtgt ggatgctgga gcggccacgg ctcatgcggt tttggcgcgg 540

ggctactcct ccctcattta catcggtgga gaaaaagagc agatttggac acggcgaggc 600

aaagacatcg tggtggtacg tccccgcaag ggtttttgca agctggccct ccagcataac 660

tgccccatcg taccggtcta cgcatttggg gaaaacgatc tgtatcgcac gttcaaccac 720

ctcaaggact tccagctgtg ggtggctagc gccttcaagc tcgcttttcc tccttgttgg 780

ggcgtcctct tcctcccctt cctcccccctc cccgtctcta tcacggtggt gatgggcgag 840

cccttgctac ccagagcaca aaaaggaagt gcgagaagga gtggtggagg aaaaggggtg 900

gagccgacga gggaggaggt ggaggagctg cacttccgat acgtggaggc cttgcagaag 960

ttgtttgacg cacacaaagt caggcaggga gggaggagcg aagaggccac cttagtggtc 1020

aaatga 1026

<210> 17
<211> 341
<212> PRT
<213> Nannochloropsis oculata

<400> 17

Met Ala Pro Ser Pro Pro Ala Pro Pro Pro Ala Pro Glu Asn Pro Tyr
1 5 10 15

Asn Leu Leu Pro Pro Lys Arg Pro Asn Pro Gln Tyr Trp Arg Tyr Ala
20 25 30

Ser Leu Ala Ala Phe Leu Leu Thr Cys Phe Leu Ala Pro Ser Ser Asn
35 40 45

Ser Trp Ala Thr Thr Leu Arg Arg Ala Cys Trp Ala Ala Tyr Trp Thr
50 55 60

Thr Tyr Leu Asp Thr Ser Tyr Lys Asp Gly Ser Arg Ala Trp Pro Trp
65 70 75 80

Phe Gln Arg Leu Arg Ile Trp Arg Met Tyr Cys Gly Tyr Leu Gln Gly
85 90 95

Lys Val Ile Cys Thr Val Pro Leu Asp Pro Ala Gln Gln Phe Ile Phe
100 105 110

Ala Ala His Pro His Gly Ile Gly Thr Trp Asn His Phe Leu Thr Met
115 120 125

Thr Asp Gly Cys Arg Phe Leu Ser Ser Ser Tyr Pro Arg Pro Arg Leu
130 135 140

Asp Leu Gly Ala Thr Val Leu Phe Phe Ile Pro Phe Leu Lys Glu Ile
145 150 155 160

Leu Leu Trp Leu Gly Cys Val Asp Ala Gly Ala Ala Thr Ala His Ala
165 170 175

Val Leu Ala Arg Gly Tyr Ser Ser Leu Ile Tyr Ile Gly Gly Glu Lys
180 185 190

Glu Gln Ile Trp Thr Arg Arg Gly Lys Asp Ile Val Val Val Arg Pro
195 200 205

Arg Lys Gly Phe Cys Lys Leu Ala Leu Gln His Asn Cys Pro Ile Val
210 215 220

```
Pro Val Tyr Ala Phe Gly Glu Asn Asp Leu Tyr Arg Thr Phe Asn His
225                 230                 235                 240

Leu Lys Asp Phe Gln Leu Trp Val Ala Ser Ala Phe Lys Leu Ala Phe
                245                 250                 255

Pro Pro Cys Trp Gly Val Leu Phe Leu Pro Phe Leu Pro Leu Pro Val
            260                 265                 270

Ser Ile Thr Val Val Met Gly Glu Pro Leu Leu Pro Arg Ala Gln Lys
        275                 280                 285

Gly Ser Ala Arg Arg Ser Gly Gly Gly Lys Gly Val Glu Pro Thr Arg
    290                 295                 300

Glu Glu Val Glu Glu Leu His Phe Arg Tyr Val Glu Ala Leu Gln Lys
305                 310                 315                 320

Leu Phe Asp Ala His Lys Val Arg Gln Gly Gly Arg Ser Glu Glu Ala
                325                 330                 335

Thr Leu Val Val Lys
            340
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<210> 18
<211> 1100
<212> DNA
<213> Nannochloropsis oculata

<400> 18

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aacgatggca ccctccccac cggccccgcc acctgcaccc gagaacccct acaacctatt      120

gccacccaag cgccccaatc cgcagtactg gcggtatgca agccttgccg ccttccttct      180

cacttgcttc ctggcccctt ccagtaactc gtgggccacc accctccgcc gcgcctgctg      240

ggcggcgtac tggacgacct acctggacac aagctataag gacggctcac gggcctggcc      300

ctggtttcag cgattgcgaa tctggcgtat gtattgcggc tatttgcagg gcaaagtcat      360

ttgcacggtg cccttggacc cggcgcagca atttatcttc gcggcccatc cccacggcat      420

tggtacctgg aaccatttcc tgaccatgac tgacggctgt cgatttctct cctcctccta      480

cccccgcccg cggctcgacc tgggtgcgac agtacttttc ttcatcccct tcttaaagga      540

aattctgctt tggctaggct gtgtggatgc tggagcggcc acggctcatg cggttttggc      600

gcggggctac tcctccctca tttacatcgg tggagaaaaa gagcagattt ggacacggcg      660

aggcaaagac atcgtggtgg tacgtccccg caagggtttt tgcaagctgg ccctccagca      720

taactgcccc atcgtaccgg tctacgcatt tggggaaaac gatctgtatc gcacgttcaa      780

ccacctcaag gacttccagc tgtgggtggc tagcgccttc aagctcgctt ttcctccttg      840

ttggggcgtc ctcttcctcc ccttcctccc cctccccgtc tctatcacgg tggtgatggg      900
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cgagcccttg ctacccagag cacaaaaagg aagtgcgaga aggagtggtg gaggaaaagg 960

ggtggagccg acgagggagg aggtggagga gctgcacttc cgatacgtgg aggccttgca 1020

gaagttgttt gacgcacaca aagtcaggca gggagggagg agcgaagagg ccaccttagt 1080

ggtcaaatga ggaaacaccc 1100

<210> 19
<211> 1206
<212> DNA
<213> Nannochloropsis oculata

<400> 19
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cagcaagagc aggcgaagct gaagcaaaaa ccttctctac tgcgggagcg caagggaggt 120

aatataacca aggagcccca gacgccctcg agtaatctga ggcctgcccg ttccccgacc 180

gaggtggact ggagctcctt ccctgagggc agctacacgc gcttcgggca tggcggggac 240

tggtggacgc taatcaaggg gacgattgcc attttgttca cgtgggggac ctggctggct 300

ggcggcttgt ctccctttg gatgacttgg ttgtatacgc acggatacaa gaggacattc 360

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tttgtccgat tcattttaaa catggctgga tattttgagg gcggtgcggc gatgtacgtc 480

gaaaactctt tcaaaggccg caatgtgaat ggtcctatca tgttggccat gcacccccat 540

ggcatcatgc ctcactcttt ccttctcaac ggtgccgggc ggatccacgc gcagaaaccg 600

gaggtattcc tccctccaca ctatcaagat atgtctctta aatcgacggg cgtggcggag 660

ccgttgttgt ttcggattcc gtttatttcg gcatttcttt atttttttgg gtgtgcggag 720

cctgcgtcga aggagatgat gcacgacatc ttggggaggc aggtgccgtt tgggatcctg 780

gtgggtggct ccgaggaaat cctcctcatg gagtaccaga aggaaaacat ctacatcctc 840

gaacgtaaag gttttattaa atacgccctt cagcatggct acaccatcgc cattggctac 900

ctcttcggcg agtccaacct ctaccacacc atcacctggg gacgcaagac ccgcctcgcc 960

ctcttcaaaa aattcaagat tccgttattt ttggcttggg gacgttggtt ctttccctta 1020

ctccctgagc gagcagcgcc tttgaatgct gtcgttggca accctattga tttgcccagg 1080

atagccaacc caagccaggc ggacattgac aaataccatg cgatgtacat tgagaaattg 1140

acagatttgt ttgaacggaa taaggcggcc tttgggtatt cagatcggac gttgaatttc 1200

ttttag 1206

<210> 20
<211> 401
<212> PRT
<213> Nannochloropsis oculata

<400> 20

Met Gly Leu Phe Gly Ser Gly Ile Lys Glu Lys Thr Glu Ala Glu Thr
1 5 10 15

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Ala Gln Val Glu Gln Gln Glu Gln Ala Lys Leu Lys Gln Lys Pro Ser
            20                  25                  30

Leu Leu Arg Glu Arg Lys Gly Gly Asn Ile Thr Lys Glu Pro Gln Thr
        35                  40                  45

Pro Ser Ser Asn Leu Arg Pro Ala Arg Ser Pro Thr Glu Val Asp Trp
    50                  55                  60

Ser Ser Phe Pro Glu Gly Ser Tyr Thr Arg Phe Gly His Gly Gly Asp
65                  70                  75                  80

Trp Trp Thr Leu Ile Lys Gly Thr Ile Ala Ile Leu Phe Thr Trp Gly
                85                  90                  95

Thr Trp Leu Ala Gly Gly Leu Ser Pro Phe Trp Met Thr Trp Leu Tyr
            100                 105                 110

Thr His Gly Tyr Lys Arg Thr Phe Tyr Ser Ile Ile Gly Pro Leu Leu
        115                 120                 125

Tyr Pro Leu Phe Leu Pro Val Pro Ala Trp Pro Gly Phe Val Arg Phe
    130                 135                 140

Ile Leu Asn Met Ala Gly Tyr Phe Glu Gly Gly Ala Ala Met Tyr Val
145                 150                 155                 160

Glu Asn Ser Phe Lys Gly Arg Asn Val Asn Gly Pro Ile Met Leu Ala
                165                 170                 175

Met His Pro His Gly Ile Met Pro His Ser Phe Leu Leu Asn Gly Ala
            180                 185                 190

Gly Arg Ile His Ala Gln Lys Pro Glu Val Phe Leu Pro Pro His Tyr
        195                 200                 205

Gln Asp Met Ser Leu Lys Ser Thr Gly Val Ala Glu Pro Leu Leu Phe
    210                 215                 220

Arg Ile Pro Phe Ile Ser Ala Phe Leu Tyr Phe Phe Gly Cys Ala Glu
225                 230                 235                 240

Pro Ala Ser Lys Glu Met Met His Asp Ile Leu Gly Arg Gln Val Pro
                245                 250                 255

Phe Gly Ile Leu Val Gly Gly Ser Glu Glu Ile Leu Leu Met Glu Tyr
            260                 265                 270

Gln Lys Glu Asn Ile Tyr Ile Leu Glu Arg Lys Gly Phe Ile Lys Tyr
        275                 280                 285
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Ala Leu Gln His Gly Tyr Thr Ile Ala Ile Gly Tyr Leu Phe Gly Glu
    290                 295                 300

Ser Asn Leu Tyr His Thr Ile Thr Trp Gly Arg Lys Thr Arg Leu Ala
305                 310                 315                 320

Leu Phe Lys Lys Phe Lys Ile Pro Leu Phe Leu Ala Trp Gly Arg Trp
                325                 330                 335

Phe Phe Pro Leu Leu Pro Glu Arg Ala Ala Pro Leu Asn Ala Val Val
            340                 345                 350

Gly Asn Pro Ile Asp Leu Pro Arg Ile Ala Asn Pro Ser Gln Ala Asp
        355                 360                 365

Ile Asp Lys Tyr His Ala Met Tyr Ile Glu Lys Leu Thr Asp Leu Phe
    370                 375                 380

Glu Arg Asn Lys Ala Ala Phe Gly Tyr Ser Asp Arg Thr Leu Asn Phe
385                 390                 395                 400

Phe
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<210> 21
<211> 1772
<212> DNA
<213> Nannochloropsis oculata

<400> 21

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acatcaacac aggtacttgc agccaccact gcagcaatta tagcaccatc acgaccacta      120

tgggtctatt tggcagcggg atcaaggaaa agacggaggc tgagaccgcg caggtggagc      180

agcaagagca ggcgaagctg aagcaaaaac cttctctact gcgggagcgc aagggaggta      240

atataaccaa ggagccccag acgccctcga gtaatctgag gcctgcccgt tccccgaccg      300

aggtggactg gagctccttc cctgagggca gctacacgcg cttcgggcat ggcggggact      360

ggtggacgct aatcaagggg acgattgcca ttttgttcac gtgggggacc tggctggctg      420

gcggcttgtc tccctttgg atgacttggt tgtatacgca cggatacaag aggacattct      480

attcgatcat aggccctttg ctttacccgc ttttcttgcc cgtgccagct tggcctggat      540

ttgtccgatt cattttaaac atggctggat attttgaggg cggtgcggcg atgtacgtcg      600

aaaactcttt caaaggccgc aatgtgaatg gtcctatcat gttggccatg cacccccatg      660

gcatcatgcc tcactctttc cttctcaacg gtgccgggcg gatccacgcg cagaaaccgg      720

aggtattcct ccctccacac tatcaagata tgtctcttaa atcgacgggc gtggcggagc      780

cgttgttgtt tcggattccg tttatttcgg catttcttta ttttttgggg tgtgcggagc      840
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ctgcgtcgaa ggagatgatg cacgacatct tggggaggca ggtgccgttt gggatcctgg 900

tgggtggctc cgaggaaatc ctcctcatgg agtaccagaa ggaaaacatc tacatcctcg 960

aacgtaaagg ttttattaaa tacgcccttc agcatggcta caccatcgcc attggctacc 1020

tcttcggcga gtccaacctc taccacacca tcacctgggg acgcaagacc cgcctcgccc 1080

tcttcaaaaa attcaagatt ccgttatttt tggcttgggg acgttggttc tttcccttac 1140

tccctgagcg agcagcgcct ttgaatgctg tcgttggcaa ccctattgat ttgcccagga 1200

tagccaaccc aagccaggcg gacattgaca aataccatgc gatgtacatt gagaaattga 1260

cagatttgtt tgaacggaat aaggcggcct ttgggtattc agatcggacg ttgaatttct 1320

tttaggtggg tgggaggaaa ggagggtaag agggagggtg ggaaggtgtg tgtaggggt 1380

gagtgttcag gcattgttgt tcaggcatgg aaagagactg acccaaccaa ctgaaaagga 1440

gatagacaag caagcacacc atggggtcaa tgatcgtgat tagagagaag atgggcaaga 1500

gggagggact gatccggtgt aaatatagac acatgactga atgaagaagc aaggagagaa 1560

tggagaggaa tcagcagcag cagcagcagc agcagcagag aacaatagct cttaaggcag 1620

cagctacaac aatcaaaaca cgaacaagag cgaaaagtcc aaacgctaag attcgacacg 1680

gagaacaaga acgaagaacg gtgatatcaa cagggaataa ttgtacgaac gaagcatgag 1740

tctagtgaaa acaacaaaaa aaaacaaaaa aa 1772

<210> 22
<211> 1173
<212> DNA
<213> Nannochloropsis oculata

<400> 22
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ggcaaggaga ccgagctttc cacgccggtc accgctccca cttcggaccg ctcgcgtacc 120

tacagtgatg gctattcgac ccccaagtcc tacacattgg aggtcgatcc caaattttat 180

aagcgggtat gcgatgctga tgacgtgtgg acacgcacac agggtgcatt tgctcttctc 240

atgctctggg gcgtctggct tgccgggtcc ttttctgtgt tttggtggcc ctatttagta 300

gtgaaggggt attatactgc tgccctagct atggcagtga tcatggcata tccgtatgtg 360

gtcaaggtca agcaaagccc ggcatttatt cgcttcatct tgagcggcgc gggatggttt 420

aagggcggga cgtgtttgta tttggaggag tcgatgaagc agatcgacac cagcgagtct 480

gtcctcctct gtcagcatcc gcatggtctc ttcacctatg gcttcatcca aaacgggtct 540

gctgcccgca tcgatgcccg caaacccgag gtttatgtgc ctgccgcatt tcgtcacatg 600

aaacccaacg ccaaggcctt cgtggaacct ttgctattca aaatcccgct tatccgtcac 660

tttatcaccg ccttcggcaa cgccgccccg gcgaccaaaa aagagatgca ccgtctcatg 720

tccactaaaa ttcccctggg gctgttaccg ggtgggtcgg aagagatcat cttaagccac 780

catggccatg agcgggtgta catcctcaaa cggaaaggct tcctcaagta cgcattacaa 840

catggctaca cgatttgcat tggttacaca ttcggggagt ccgactcgta ccgcaccttg 900

gactggggcg tgaagtttcg tacgtggtac ctgaagacct tccgcgttcc actctttgcg 960

tgctggggga cgtggtggtg ccccctcttg ccacggggga aggtggcgct tgagacagtc 1020

gttgggaacc catttcggtt gcccaagatt gtagatccga gccaggagga tattgataag 1080

tggcatgcgg tgtatgtgca aaaacttgta gatttgtttg atcggaacaa ggccaagttc 1140

gggtatgggg acagggagct ggatttcttt tag 1173

<210> 23
<211> 390
<212> PRT
<213> Nannochloropsis oculata

<400> 23

Met Leu Ser Ile Pro Glu Ser Ser Ser Pro Leu Ser Asp Arg Thr Leu
1 5 10 15

Val Lys Asn Gly Gly Lys Glu Thr Glu Leu Ser Thr Pro Val Thr Ala
20 25 30

Pro Thr Ser Asp Arg Ser Arg Thr Tyr Ser Asp Gly Tyr Ser Thr Pro
35 40 45

Lys Ser Tyr Thr Leu Glu Val Asp Pro Lys Phe Tyr Lys Arg Val Cys
50 55 60

Asp Ala Asp Asp Val Trp Thr Arg Thr Gln Gly Ala Phe Ala Leu Leu
65 70 75 80

Met Leu Trp Gly Val Trp Leu Ala Gly Ser Phe Ser Val Phe Trp Trp
85 90 95

Pro Tyr Leu Val Val Lys Gly Tyr Tyr Thr Ala Ala Leu Ala Met Ala
100 105 110

Val Ile Met Ala Tyr Pro Tyr Val Val Lys Val Lys Gln Ser Pro Ala
115 120 125

Phe Ile Arg Phe Ile Leu Ser Gly Ala Gly Trp Phe Lys Gly Gly Thr
130 135 140

Cys Leu Tyr Leu Glu Glu Ser Met Lys Gln Ile Asp Thr Ser Glu Ser
145 150 155 160

Val Leu Leu Cys Gln His Pro His Gly Leu Phe Thr Tyr Gly Phe Ile
165 170 175

Gln Asn Gly Ser Ala Ala Arg Ile Asp Ala Arg Lys Pro Glu Val Tyr
180 185 190

Val Pro Ala Ala Phe Arg His Met Lys Pro Asn Ala Lys Ala Phe Val

195 200 205

Glu Pro Leu Leu Phe Lys Ile Pro Leu Ile Arg His Phe Ile Thr Ala
210 215 220

Phe Gly Asn Ala Ala Pro Ala Thr Lys Lys Glu Met His Arg Leu Met
225 230 235 240

Ser Thr Lys Ile Pro Leu Gly Leu Leu Pro Gly Gly Ser Glu Glu Ile
245 250 255

Ile Leu Ser His His Gly His Glu Arg Val Tyr Ile Leu Lys Arg Lys
260 265 270

Gly Phe Leu Lys Tyr Ala Leu Gln His Gly Tyr Thr Ile Cys Ile Gly
275 280 285

Tyr Thr Phe Gly Glu Ser Asp Ser Tyr Arg Thr Leu Asp Trp Gly Val
290 295 300

Lys Phe Arg Thr Trp Tyr Leu Lys Thr Phe Arg Val Pro Leu Phe Ala
305 310 315 320

Cys Trp Gly Thr Trp Trp Cys Pro Leu Leu Pro Arg Gly Lys Val Ala
325 330 335

Leu Glu Thr Val Val Gly Asn Pro Phe Arg Leu Pro Lys Ile Val Asp
340 345 350

Pro Ser Gln Glu Asp Ile Asp Lys Trp His Ala Val Tyr Val Gln Lys
355 360 365

Leu Val Asp Leu Phe Asp Arg Asn Lys Ala Lys Phe Gly Tyr Gly Asp
370 375 380

Arg Glu Leu Asp Phe Phe
385 390

<210> 24
<211> 1239
<212> DNA
<213> Nannochloropsis oculata

<400> 24
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ggtagaatgt tgagtatccc cgagtcgtcc tcgcccctct cggaccggac tctggtgaag 120

aatggaggca aggagaccga gctttccacg ccggtcaccg ctcccacttc ggaccgctcg 180

cgtacctaca gtgatggcta ttcgaccccc aagtcctaca cattggaggt cgatcccaaa 240

ttttataagc gggtatgcga tgctgatgac gtgtggacac gcacacaggg tgcatttgct 300

cttctcatgc tctggggcgt ctggcttgcc gggtcctttt ctgtgttttg gtggccctat 360

ttagtagtga aggggtatta tactgctgcc ctagctatgg cagtgatcat ggcatatccg 420

tatgtggtca aggtcaagca aagcccggca tttattcgct tcatcttgag cggcgcggga 480

tggtttaagg gcgggacgtg tttgtatttg gaggagtcga tgaagcagat cgacaccagc 540

gagtctgtcc tcctctgtca gcatccgcat ggtctcttca cctatggctt catccaaaac 600

gggtctgctg cccgcatcga tgcccgcaaa cccgaggttt atgtgcctgc cgcatttcgt 660

cacatgaaac ccaacgccaa ggccttcgtg gaacctttgc tattcaaaat cccgcttatc 720

cgtcacttta tcaccgcctt cggcaacgcc gccccggcga ccaaaaaaga gatgcaccgt 780

ctcatgtcca ctaaaattcc cctggggctg ttaccgggtg ggtcggaaga gatcatctta 840

agccaccatg gccatgagcg ggtgtacatc ctcaaacgga aaggcttcct caagtacgca 900

ttacaacatg gctacacgat ttgcattggt tacacattcg gggagtccga ctcgtaccgc 960

accttggact ggggcgtgaa gtttcgtacg tggtacctga agaccttccg cgttccactc 1020

tttgcgtgct gggggacgtg gtggtgcccc ctcttgccac gggggaaggt ggcgcttgag 1080

acagtcgttg ggaacccatt tcggttgccc aagattgtag atccgagcca ggaggatatt 1140

gataagtggc atgcggtgta tgtgcaaaaa cttgtagatt tgtttgatcg gaacaaggcc 1200

aagttcgggt atggggacag ggagctggat ttcttttag 1239

<210> 25
<211> 1089
<212> DNA
<213> Nannochloropsis oculata

<400> 25
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tccccctcca agacctcgcc ccccgccgtt caatacaaag cagggaatgg caaggtgatc 120

acggtggcca tggccgagca agacgacggg aacatgggca ttttccgcga gtgttgtgcg 180

atggtgacaa tggggataat catgtcgtgg tactacatcg tcgtcgttct ctccctcctg 240

tgcttggtgg ggatctcctt cttccctgcc tggcgggcgg tggcggcgac ggtttttgta 300

ctcatgtgga gtgcggcgct tttgccgctc gactaccagg ggtgggacgc tttctgcaac 360

tcatgtatct tcaggctgtg gcgggactac ttccactacg aatacgtcct ggaagaaatg 420

atcgacccca acaagcgcta cctcttcgct gagatgcccc acggaatctt cccctgggga 480

gaggtgattt ccatttctat caccaagcag cttttccccg ggagccgcgt cggctccatt 540

ggtgcgagtg tcatcttcct ccttccgggc ctccggcact tcttcgcctg gatcgggtgt 600

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acggtgggag gggtcgccga gatgtttctg gttggaggag agaaggagcg gctctaccta 720

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gtcgtgcaga atgcagagcc gaccaaggag gagatcgcgg cgacgcacgc actcttttgc 1020

gagaaggtgg aggagcttta ctacaaattc aggccggaat gggagacgcg cccgttgtcc 1080

attgagtaa 1089

<210> 26
<211> 362
<212> PRT
<213> Nannochloropsis oculata

<400> 26

Met Thr Pro Gln Ala Asp Ile Thr Ser Lys Thr Thr Ser Asn Pro Lys
1 5 10 15

Thr Ala Ala Ser Ser Pro Ser Lys Thr Ser Pro Pro Ala Val Gln Tyr
20 25 30

Lys Ala Gly Asn Gly Lys Val Ile Thr Val Ala Met Ala Glu Gln Asp
35 40 45

Asp Gly Asn Met Gly Ile Phe Arg Glu Cys Cys Ala Met Val Thr Met
50 55 60

Gly Ile Ile Met Ser Trp Tyr Tyr Ile Val Val Val Leu Ser Leu Leu
65 70 75 80

Cys Leu Val Gly Ile Ser Phe Phe Pro Ala Trp Arg Ala Val Ala Ala
85 90 95

Thr Val Phe Val Leu Met Trp Ser Ala Ala Leu Leu Pro Leu Asp Tyr
100 105 110

Gln Gly Trp Asp Ala Phe Cys Asn Ser Cys Ile Phe Arg Leu Trp Arg
115 120 125

Asp Tyr Phe His Tyr Glu Tyr Val Leu Glu Glu Met Ile Asp Pro Asn
130 135 140

Lys Arg Tyr Leu Phe Ala Glu Met Pro His Gly Ile Phe Pro Trp Gly
145 150 155 160

Glu Val Ile Ser Ile Ser Ile Thr Lys Gln Leu Phe Pro Gly Ser Arg
165 170 175

Val Gly Ser Ile Gly Ala Ser Val Ile Phe Leu Leu Pro Gly Leu Arg
180 185 190

His Phe Phe Ala Trp Ile Gly Cys Arg Pro Ala Ser Pro Glu Asn Ile
195 200 205

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Lys Lys Ile Phe Asp Asp Gly Gln Asp Cys Ala Val Thr Val Gly Gly
    210                 215                 220

Val Ala Glu Met Phe Leu Val Gly Gly Glu Lys Glu Arg Leu Tyr Leu
225                 230                 235                 240

Lys Lys His Lys Gly Phe Val Arg Glu Ala Met Lys Asn Gly Ala Asp
                245                 250                 255

Leu Val Pro Val Phe Cys Phe Gly Asn Ser Lys Leu Phe Asn Val Val
            260                 265                 270

Gly Glu Ser Ser Arg Val Ser Met Gly Leu Met Lys Arg Leu Ser Arg
        275                 280                 285

Arg Leu Lys Ala Ser Val Leu Ile Phe Tyr Gly Arg Leu Phe Leu Pro
    290                 295                 300

Ile Pro Ile Arg His Pro Leu Leu Phe Val Val Gly Lys Pro Leu Pro
305                 310                 315                 320

Val Val Gln Asn Ala Glu Pro Thr Lys Glu Glu Ile Ala Ala Thr His
                325                 330                 335

Ala Leu Phe Cys Glu Lys Val Glu Glu Leu Tyr Tyr Lys Phe Arg Pro
            340                 345                 350

Glu Trp Glu Thr Arg Pro Leu Ser Ile Glu
        355                 360
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<210> 27
<211> 1609
<212> DNA
<213> Nannochloropsis oculata

<400> 27

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caagacgaca tccaacccca agacggctgc atcctccccc tccaagacct cgccccccgc      180

cgttcaatac aaagcaggga atggcaaggt gatcacggtg gccatggccg agcaagacga      240

cgggaacatg ggcattttcc gcgagtgttg tgcgatggtg acaatgggga taatcatgtc      300

gtggtactac atcgtcgtcg ttctctccct cctgtgcttg gtggggatct ccttcttccc      360

tgcctggcgg gcggtggcgg cgacggtttt tgtactcatg tggagtgcgg cgcttttgcc      420

gctcgactac caggggtggg acgctttctg caactcatgt atcttcaggc tgtggcggga      480

ctacttccac tacgaatacg tcctggaaga aatgatcgac cccaacaagc gctacctctt      540

cgctgagatg ccccacggaa tcttcccctg gggagaggtg atttccattt ctatcaccaa      600

gcagcttttc cccgggagcc gcgtcggctc cattggtgcg agtgtcatct tcctccttcc      660
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gggcctccgg cacttcttcg cctggatcgg gtgtcggccc gcgagcccgg agaatatcaa 720

aaagattttt gatgatgggc aggattgtgc cgtgacggtg ggaggggtcg ccgagatgtt 780

tctggttgga ggagagaagg agcggctcta cctaaaaaag cacaagggtt tcgttcgaga 840

ggccatgaag aacggcgcgg acctggtccc tgtcttctgc ttcggcaaca gcaagttgtt 900

caatgtggtg ggggagagca gtcgggtgtc catgggcctg atgaagcgtc tctcgaggag 960

gctcaaagcc agcgtcctca ttttctacgg ccgtctcttc ctacccattc cgatccgcca 1020

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gattagaaga cgagacttgg atacgatgaa gggtgaagaa gaaatttaaa acaattttga 1380

gattttattc atgtctgagg aataaatgta gatgttagaa aatttgaggt agttctcggt 1440

acttgtcccc tatcatccgt gtttagtaac gaggtacatc cgtgcgacgg gtcggtggaa 1500

gtagccagcg tcatcagaga gaggtctcac acacgatcgt gtgtccttgc acatgtcttt 1560

tccatttaac acgaattact ttttttaaa aaaataataa aaaaaaata 1609

<210> 28
<211> 1464
<212> DNA
<213> Nannochloropsis oculata

<400> 28
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gctcccgcca cgtccaaagc cacgacaagc agcataaagg agattgggaa gccctcattg 180

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gccgcagtag cagcaggagc actggagggg gctgcagcag gcgccgtgac agcaccaccc 300

accgaccaat ctccgaagaa gcagtacggg cagggtggta ctggggagcg agggaaggag 360

gcagaaggtg gacgagaacg aagtggaagc gtcggcaacc ttttactgtc atcaattaat 420

tcgttttcaa gctgcacgtc cctatccttt ttggccggcg aggacgagac cccgtctcct 480

cccgagacag ggcctgctgg gattgatttc tcgacaccgg ctcatccgac catgcaactt 540

gtggacttca tcatcacttt tctcttggtg cattatattc aagtcttcta ctccctagtc 600

ctcctcttca tctacctcgt caagcacggt cacagatggc cgtacctcct cgctgccatc 660

tacgcccctt cgtacttcat tcctttacag cgattgggcg gatggccgtt caaaggattc 720

atgcgtcggc ccttttggcg gtgtgtccaa aggaccttag ctctccaggt ggaaagagag 780

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ctgggtgggg tggatgcagg cagggcatca gcagcacggg cactcacaga cggctactcc 1020

gtctctcttt atccgggggg aagcaaggaa atctacacca ctgatcccta cactcctgaa 1080

acgaccctgg tcctgaaaat ccgcaaaggc ttcattcgca tggccctccg ctatggctgt 1140

ccactcgtgc ctgtgtacac gtttggagaa aaatacgcct accatcggct agggccggcc 1200

acgggctttg cgcgctggct gttggcagtg ctgaaagtcc ctttcttgat cttttgggga 1260

cgatggggca cattcatgcc gctcaaggag acgcaggtgt cagtggtggt gggcaagcca 1320

ctgcgcgtgc ccaaaatcga tggagatcct gcccctgagg tggtggagga atggttgcac 1380

agatactgcg acgaagtcca ggcgttgttc cagcgacaca agaacaaata cgcaaagcct 1440

gaggagttca ttgcgatcgc ctaa 1464

<210> 29
<211> 487
<212> PRT
<213> Nannochloropsis oculata

<400> 29

Met Ala Tyr Leu Phe Arg Arg Arg Ser Lys Gly Glu Gly Asn Ser Thr
1 5 10 15

Ser Ser Ser Cys Ser Ser Leu Ser Glu Asp Asn Lys Gly Thr Ser Ile
20 25 30

His Ser Ser Glu Ile Glu Pro Arg Ala Pro Ala Thr Ser Lys Ala Thr
35 40 45

Thr Ser Ser Ile Lys Glu Ile Gly Lys Pro Ser Leu Pro Thr Ala Ala
50 55 60

His Leu Ser Pro Pro Ser Ile Ser Lys Ala Asp Arg Asn Phe Ala Ile
65 70 75 80

Ala Ala Val Ala Ala Gly Ala Leu Glu Gly Ala Ala Ala Gly Ala Val
85 90 95

Thr Ala Pro Pro Thr Asp Gln Ser Pro Lys Lys Gln Tyr Gly Gln Gly
100 105 110

Gly Thr Gly Glu Arg Gly Lys Glu Ala Glu Gly Gly Arg Glu Arg Ser
115 120 125

Gly Ser Val Gly Asn Leu Leu Leu Ser Ser Ile Asn Ser Phe Ser Ser
130 135 140

Cys Thr Ser Leu Ser Phe Leu Ala Gly Glu Asp Glu Thr Pro Ser Pro
145 150 155 160

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Pro Glu Thr Gly Pro Ala Gly Ile Asp Phe Ser Thr Pro Ala His Pro
                165                 170                 175

Thr Met Gln Leu Val Asp Phe Ile Ile Thr Phe Leu Leu Val His Tyr
            180                 185                 190

Ile Gln Val Phe Tyr Ser Leu Val Leu Leu Phe Ile Tyr Leu Val Lys
        195                 200                 205

His Gly His Arg Trp Pro Tyr Leu Leu Ala Ala Ile Tyr Ala Pro Ser
    210                 215                 220

Tyr Phe Ile Pro Leu Gln Arg Leu Gly Gly Trp Pro Phe Lys Gly Phe
225                 230                 235                 240

Met Arg Arg Pro Phe Trp Arg Cys Val Gln Arg Thr Leu Ala Leu Gln
                245                 250                 255

Val Glu Arg Glu Val Glu Leu Arg Pro Asp Glu Gln Tyr Ile Phe Gly
            260                 265                 270

Trp His Pro His Gly Ile Leu Leu Leu Ser Arg Phe Ala Ile Tyr Gly
        275                 280                 285

Gly Leu Trp Glu Lys Leu Phe Pro Gly Ile His Phe Lys Thr Leu Ala
    290                 295                 300

Ala Ser Pro Leu Phe Trp Ile Pro Pro Ile Arg Glu Val Ser Ile Leu
305                 310                 315                 320

Leu Gly Gly Val Asp Ala Gly Arg Ala Ser Ala Ala Arg Ala Leu Thr
                325                 330                 335

Asp Gly Tyr Ser Val Ser Leu Tyr Pro Gly Gly Ser Lys Glu Ile Tyr
            340                 345                 350

Thr Thr Asp Pro Tyr Thr Pro Glu Thr Thr Leu Val Leu Lys Ile Arg
        355                 360                 365

Lys Gly Phe Ile Arg Met Ala Leu Arg Tyr Gly Cys Pro Leu Val Pro
    370                 375                 380

Val Tyr Thr Phe Gly Glu Lys Tyr Ala Tyr His Arg Leu Gly Pro Ala
385                 390                 395                 400

Thr Gly Phe Ala Arg Trp Leu Leu Ala Val Leu Lys Val Pro Phe Leu
                405                 410                 415

Ile Phe Trp Gly Arg Trp Gly Thr Phe Met Pro Leu Lys Glu Thr Gln
            420                 425                 430
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Val Ser Val Val Val Gly Lys Pro Leu Arg Val Pro Lys Ile Asp Gly
435 440 445

Asp Pro Ala Pro Glu Val Val Glu Glu Trp Leu His Arg Tyr Cys Asp
450 455 460

Glu Val Gln Ala Leu Phe Gln Arg His Lys Asn Lys Tyr Ala Lys Pro
465 470 475 480

Glu Glu Phe Ile Ala Ile Ala
485

<210> 30
<211> 1682
<212> DNA
<213> Nannochloropsis oculata

<400> 30
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caaaggcgag ggcaacagca ctagcagcag ctgctcttct ctgtcggaag ataataaggg 180

cacgtccatc cactcttccg aaatcgagcc gcgcgctccc gccacgtcca aagccacgac 240

aagcagcata aaggagattg ggaagccctc attgcccacc gccgcacatt tatcaccacc 300

cagcataagc aaggcagata gaaatttcgc cattgccgca gtagcagcag gagcactgga 360

gggggctgca gcaggcgccg tgacagcacc acccaccgac caatctccga agaagcagta 420

cgggcagggt ggtactgggg agcgagggaa ggaggcagaa ggtggacgag aacgaagtgg 480

aagcgtcggc aacctttttac tgtcatcaat taattcgttt tcaagctgca cgtccctatc 540

ctttttggcc ggcgaggacg agaccccgtc tcctcccgag acagggcctg ctgggattga 600

tttctcgaca ccggctcatc cgaccatgca acttgtggac ttcatcatca cttttctctt 660

ggtgcattat attcaagtct tctactccct agtcctcctc ttcatctacc tcgtcaagca 720

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ggaaatctac accactgatc cctacactcc tgaaacgacc ctggtcctga aaatccgcaa 1200

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tcctgcccct gaggtggtgg aggaatggtt gcacagatac tgcgacgaag tccaggcgtt 1500

gttccagcga cacaagaaca aatacgcaaa gcctgaggag ttcattgcga tcgcctaaaa 1560

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accaaccacg cgacatgagg ggacggaagg agctggatgc ggtgtggttt gtctgttcag 1680

ga 1682

<210> 31
<211> 1539
<212> DNA
<213> Nannochloropsis oculata

<400> 31
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gtcagcggcc aaaggtgtgc acgagctttg cgtccttccc caggggtgat tcgaaggaaa 180

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cctgtcgatc ctattatcaa tgccgtggtg cttttcgagg gggaggcgcc cacgcgtgcg 300

gcggtggaat cggccatctt gccgctcttt gaattcgaac ggtttcgctc ccggaaggtt 360

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attgaagact ctttcaaggg tgccagcatc gatgacttgt ttcttcgcct ggaggtgtgg 480

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ccgatcgggg agaagtttcg ccggcgggaa gcagggttca agccgacttt ccgcacccct 720

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gacgtggggt tgacgattcc ggataaaaag aatattacct ttacggggcg tcggtgcatt 840

gtgcgtatcc ccaccgtgaa gctttcgttc atcaagagca ttaaaaatgc ggcgaatgtg 900

actgtgaacg atgtggtgat gagcgcggtt gctggggccg tgcatcgatt tcgttgcgcg 960

caaaaagatc ctgcaatgct cgacccttta tcccattgta aagtccgtac acgcgctttg 1020

atgcctgtgg ctttgccccg ggaggaggga gatcctgtca aggctttgcg aaacaagtgg 1080

agttttgctt ccgtggcgat gcccgtgggg gtcaagggga gtttggaacg cttgcatgca 1140

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gaggctaacc taggggcacg cttgccgtgg acagtggcaa aacaaaccgc gtttgactcg 1260

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gccggtcggg aagtgtcggg actgtatatg gcgtttgcga atttgattcc tcaggtgggc 1380

gctctgtcct tgaacggcaa gatcttcacc tgtctggtgc tggacgacga ggtcacgccg 1440

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gggctggaaa atgtaaagaa ggaggatatt ttcgggtga 1539

<210> 32
<211> 512
<212> PRT
<213> Nannochloropsis oculata

<400> 32

Met Pro Phe Gly Arg Ala Ala Ser Ala Trp Ile Ser Ala Ser Ala Leu
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Leu Pro Ala Leu Ala Asp Pro Thr Phe Leu Cys Gly Thr Ala Ile Val
20 25 30

Gly Leu Val Val Met Tyr Tyr Ile Val Ser Gly Gln Arg Cys Ala Arg
35 40 45

Ala Leu Arg Pro Ser Pro Gly Val Ile Arg Arg Lys Met Ser Phe Cys
50 55 60

Ser Ala Ala Cys Ala Asp Gly Pro Met Pro Glu His Ala Lys Met Asn
65 70 75 80

Pro Val Asp Pro Ile Ile Asn Ala Val Val Leu Phe Glu Gly Glu Ala
85 90 95

Pro Thr Arg Ala Ala Val Glu Ser Ala Ile Leu Pro Leu Phe Glu Phe
100 105 110

Glu Arg Phe Arg Ser Arg Lys Val Lys Ile Gly Asp Asp Trp Tyr Trp
115 120 125

Glu Val Leu Pro Ser Phe Asp Ala Arg Thr His Val Ile Glu Asp Ser
130 135 140

Phe Lys Gly Ala Ser Ile Asp Asp Leu Phe Leu Arg Leu Glu Val Trp
145 150 155 160

Ser Gln Lys Pro Leu His Val Pro Val Asp Gly Pro Ala Phe Glu Phe
165 170 175

Ala Leu Leu Arg Asn Gln Asp Lys Lys Gly Pro Ser Ala Val Ile Cys
180 185 190

Arg Ile Asn His Ala Ile Gly Asp Gly Val Ser Leu Ala Lys Leu Ile
195 200 205

Pro His Val Phe Lys Asp Ile Asp Gly Gln Ser Leu Pro Ile Gly Glu
210 215 220

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Lys Phe Arg Arg Arg Glu Ala Gly Phe Lys Pro Thr Phe Arg Thr Pro
225                 230                 235                 240

Phe Thr Leu Leu Ala Ser Leu Phe Lys Val Leu Gly Thr Pro Thr Thr
                245                 250                 255

Ala Phe Asp Thr Asp Val Gly Leu Thr Ile Pro Asp Lys Lys Asn Ile
            260                 265                 270

Thr Phe Thr Gly Arg Arg Cys Ile Val Arg Ile Pro Thr Val Lys Leu
        275                 280                 285

Ser Phe Ile Lys Ser Ile Lys Asn Ala Ala Asn Val Thr Val Asn Asp
    290                 295                 300

Val Val Met Ser Ala Val Ala Gly Ala Val His Arg Phe Arg Cys Ala
305                 310                 315                 320

Gln Lys Asp Pro Ala Met Leu Asp Pro Leu Ser His Cys Lys Val Arg
                325                 330                 335

Thr Arg Ala Leu Met Pro Val Ala Leu Pro Arg Glu Glu Gly Asp Pro
            340                 345                 350

Val Lys Ala Leu Arg Asn Lys Trp Ser Phe Ala Ser Val Ala Met Pro
        355                 360                 365

Val Gly Val Lys Gly Ser Leu Glu Arg Leu His Ala Ala Asn Ala Thr
    370                 375                 380

Met Thr Ala Leu Lys Asn Ser Pro Ile Val Ile Val Gln Asn Met Val
385                 390                 395                 400

Glu Ala Asn Leu Gly Ala Arg Leu Pro Trp Thr Val Ala Lys Gln Thr
                405                 410                 415

Ala Phe Asp Ser Phe Val Arg His Thr Phe Val Phe Ser Asn Val Pro
            420                 425                 430

Gly Pro Asn Met Pro Ile Thr Phe Ala Gly Arg Glu Val Ser Gly Leu
        435                 440                 445

Tyr Met Ala Phe Ala Asn Leu Ile Pro Gln Val Gly Ala Leu Ser Leu
    450                 455                 460

Asn Gly Lys Ile Phe Thr Cys Leu Val Leu Asp Asp Glu Val Thr Pro
465                 470                 475                 480

Gly Ala Arg Glu Leu Gly Glu His Phe Ile Asp Glu Leu Met Asp Leu
                485                 490                 495
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Ala Arg Arg Thr Gly Leu Glu Asn Val Lys Lys Glu Asp Ile Phe Gly
500 505 510

<210> 33
<211> 1904
<212> DNA
<213> Nannochloropsis oculata

<400> 33
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ccacacagac gccccagctt caactctcca cacacgattt gccagtgagg gtcgtgcacc 120

ctccgcaacc acgagccttt tccacagtag tcatcctgcc catcacgctt aaaatcatgc 180

cttttggacg ggctgcatca gcctggattt cggcctcagc attgttgcca gccttggcgg 240

acccaacttt cctttgcggc accgccatcg tgggcctcgt cgttatgtac tacattgtca 300

gcggccaaag gtgtgcacga gctttgcgtc cttccccagg ggtgattcga aggaaaatga 360

gtttttgttc ggcggcctgt gcggatggtc ccatgcctga gcacgccaag atgaaccctg 420

tcgatcctat tatcaatgcc gtggtgcttt tcgaggggga ggcgcccacg cgtgcggcgg 480

tggaatcggc catcttgccg ctctttgaat tcgaacggtt tcgctcccgg aaggttaaga 540

ttggtgatga ttggtattgg gaagtgctgc cttcctttga cgctaggacg catgtgattg 600

aagactcttt caagggtgcc agcatcgatg acttgtttct tcgcctggag gtgtggtccc 660

agaaacccct gcatgtaccg gtggacgggc ccgcctttga atttgctttg cttcggaatc 720

aggataagaa ggggccctct gctgtgattt gtcgtatcaa ccatgcgatt ggtgatggtg 780

tctctctggc caagttgatc ccccacgtgt tcaaggacat tgacggccag tcactgccga 840

tcggggagaa gtttcgccgg cgggaagcag ggttcaagcc gactttccgc acccctttta 900

ccttgctggc ttcgcttttc aaggtattgg gtacgcctac tacggcgttt gatactgacg 960

tggggttgac gattccggat aaaaagaata ttacctttac ggggcgtcgg tgcattgtgc 1020

gtatccccac cgtgaagctt tcgttcatca agagcattaa aaatgcggcg aatgtgactg 1080

tgaacgatgt ggtgatgagc gcggttgctg gggccgtgca tcgatttcgt tgcgcgcaaa 1140

aagatcctgc aatgctcgac cctttatccc attgtaaagt ccgtacacgc gctttgatgc 1200

ctgtggcttt gccccgggag gagggagatc ctgtcaaggc tttgcgaaac aagtggagtt 1260

ttgcttccgt ggcgatgccc gtgggggtca aggggagttt ggaacgcttg catgcagcga 1320

atgccacgat gactgcgttg aaaaacagtc cgatagtgat cgtgcagaat atggtggagg 1380

ctaacctagg ggcacgcttg ccgtggacag tggcaaaaca aaccgcgttt gactcgtttg 1440

tgaggcacac gtttgtgttt agcaatgtac cgggtccgaa catgcctata acatttgccg 1500

gtcgggaagt gtcgggactg tatatggcgt ttgcgaattt gattcctcag gtgggcgctc 1560

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cacgtgaact aggagagcat tttattgacg agttgatgga cttggctcga aggacggggc 1680

tggaaaatgt aaagaaggag gatattttcg ggtgagaagc ctagaggaga gagggataga 1740

aggagggaag gatggagatg gtttttgtac atgcgcgtgt cggtggctgc cgcggctgtc 1800

attggtgagg cgatcggtag ggtaaataga atgaactcat aagagaatga agagtgagaa 1860

agaagagcat ccgtaagcgg gaaacaaaaa aaaaaaaaaa aaaa 1904

<210> 34
<211> 1083
<212> DNA
<213> Nannochloropsis oculata

<400> 34
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cccgtgacgg aggccaaggc aggtgatttg gggtttgggg atgttgagtc catgacggcc 180

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acagatgtcg cgctcaggct gcctttattt cgacattact taggcgccat tggtactatt 600

gccgcgactg ggcacgtggc gaagcagtac ctcgacgaag ggtggtcaat aggcatatct 660

tcgggcggag tcgcggaaat tttcgaggta aataataagg atgaagtggt gttgatgaag 720

gagaggaagg gctttgtgaa gctcgccctt cgcacgggaa ctccgctggt ggcttgttat 780

atatttggga ataccaagct gttgtcggcg tggtatgatg atggaggtgt gttgcagggt 840

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tga 1083

<210> 35
<211> 360
<212> PRT
<213> Nannochloropsis oculata

<400> 35

Met Ala Lys Ala Asn Phe Pro Pro Ala Ala Arg Tyr Val Asn Met Thr
1 5 10 15

Gln Val Tyr Ala Thr Gly Ala His Asn Met Pro Asp Glu Asp Arg Val
20 25 30

Lys Val Met Asn Gly Leu Ser Lys Pro Val Thr Glu Ala Lys Ala Gly

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        35                  40                  45

Asp Leu Gly Phe Gly Asp Val Glu Ser Met Thr Ala Trp Glu Glu Phe
    50                  55                  60

Val Ala Ala Met Phe Leu Leu Ile Ile Val Gly Ser Met Leu Trp Ile
65                  70                  75                  80

Pro Ile Ala Val Val Gly Phe Val Leu Cys Val Arg Ser Ala Val Ala
                85                  90                  95

Trp Val Val Met Leu Ile Val Phe Phe Ala Leu Ser Leu His Pro Val
            100                 105                 110

Pro Arg Ile His Asp Met Val His Ser Pro Leu Asn His Phe Ile Phe
        115                 120                 125

Lys Tyr Phe Ser Leu Lys Met Ala Ser Asp Ala Pro Leu Asp Ser Ala
    130                 135                 140

Gly Arg Tyr Ile Phe Val Ala Pro Pro His Gly Val Leu Pro Met Gly
145                 150                 155                 160

Asn Leu Met Thr Val His Ala Met Lys Ala Cys Gly Gly Leu Glu Phe
                165                 170                 175

Arg Gly Leu Thr Thr Asp Val Ala Leu Arg Leu Pro Leu Phe Arg His
            180                 185                 190

Tyr Leu Gly Ala Ile Gly Thr Ile Ala Ala Thr Gly His Val Ala Lys
        195                 200                 205

Gln Tyr Leu Asp Glu Gly Trp Ser Ile Gly Ile Ser Ser Gly Gly Val
    210                 215                 220

Ala Glu Ile Phe Glu Val Asn Asn Lys Asp Glu Val Val Leu Met Lys
225                 230                 235                 240

Glu Arg Lys Gly Phe Val Lys Leu Ala Leu Arg Thr Gly Thr Pro Leu
                245                 250                 255

Val Ala Cys Tyr Ile Phe Gly Asn Thr Lys Leu Leu Ser Ala Trp Tyr
            260                 265                 270

Asp Asp Gly Gly Val Leu Gln Gly Leu Ser Arg Tyr Leu Lys Cys Gly
        275                 280                 285

Val Leu Pro Leu Trp Gly Arg Phe Gly Leu Pro Leu Met His Arg His
    290                 295                 300

Pro Val Leu Gly Ala Met Ala Lys Pro Ile Val Val Pro Lys Val Glu
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305 310 315 320

Gly Glu Pro Thr Gln Glu Met Ile Asp Asp Tyr His Asn Leu Phe Cys
325 330 335

Gln Thr Leu Val Asp Leu Phe Asp Arg Tyr Lys Gly Leu Tyr Gly Trp
340 345 350

Pro Asp Lys Lys Leu Leu Ile Lys
355 360

<210> 36
<211> 1362
<212> DNA
<213> Nannochloropsis oculata

<400> 36
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tgacgcaggt ctatgcgaca ggcgctcaca atatgccgga cgaggaccgc gtcaaggtca 180

tgaacgggct gtccaagccc gtgacggagg ccaaggcagg tgatttgggg tttggggatg 240

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agttccgtgg gctgacgaca gatgtcgcgc tcaggctgcc tttatttcga cattacttag 660

gcgccattgg tactattgcc gcgactgggc acgtggcgaa gcagtacctc gacgaagggt 720

ggtcaatagg catatcttcg ggcggagtcg cggaaatttt cgaggtaaat aataaggatg 780

aagtggtgtt gatgaaggag aggaagggct ttgtgaagct cgcccttcgc acgggaactc 840

cgctggtggc ttgttatata tttgggaata ccaagctgtt gtcggcgtgg tatgatgatg 900

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tgcagcacaa caaatcaaag aaagcatgaa aacacaatcc aa 1362

<210> 37
<211> 1695
<212> DNA
<213> Nannochloropsis oculata

<400> 37
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tttgtgatct cgacgtttgc tgtggggttt gagttgtttg ttggagcggc ggtggtggcg 120

ggcggcttct ttttggtctc ggaagtgttg atgattgtga gtttgcattt ttatatgcct 180

acgacgacca cgactgtgac aacgaccggg ttggcggtga tggaggagaa ggtggaggag 240

gtggaggaga tgatggtggg gaaggaggga gtgggggaag aggacgagga gatggtggag 300

gaaaaggtgg acgtgacgac agcggcgacg acgaacgcac tcttaagaac cgaaaagcag 360

cggctgctct tggcgaaaga gagtgctacg accactacta ctaccgcgac tgtgaccacg 420

gggcagacca gcaagacgtc tacttcattt atgcctgtcc gggtcgacga ggcttccctt 480

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tggttcggcg ctttttgttg tctgacctca gcggtcgttt taaacgccta tgtcaaaacc 660

acgttggcca aagctgggaa tcgtatttcc tccttccagc gctccctcct taatgtcctc 720

cccacgctca tttatgccgc gccgggtctt atttgctttt ttgcgtggag tcaacaccaa 780

ggtgggaggg aggacgggaa ggagcgcgcg gtgactgcgt tcccggcttg ggcggcgctc 840

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ttagaaggga tagatcaata ttttcaggcg aagttggtct tcatggaccc gaaactggat 1020

ctgaagggga aaccgcatgt gtttgcgttt cacccacacg gagtccagcc gtttacgacg 1080

ttttggattc agctttcgcg ggcctggagg gagggagtgg ggaagggaca gagattctgt 1140

gtgatgactg cgagtgttat gcattatgtg ccgttaatgc gcgatatatt acagtggctc 1200

gggggggcggg aagtgagcag ggaagccatt tcgtacgcac tggaccgtaa acagtcagta 1260

ttgttggttc caggcggaca acaagagatg atggagtccc aatctcagat gggcgagatt 1320

cggatcatta cgaagcacgt cggcttcatt agattagcac tccagacagg cgcgccgctc 1380

gtgcctgtgc tctcatttgg cgaagttgaa gtgatggatt ttgtccggta cccgcgtcta 1440

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ttggtcctgt tgtga 1695

<210> 38
<211> 564
<212> PRT

<213> Nannochloropsis oculata

<400> 38

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Met Leu Leu Gln Gly Leu Ser Trp Ser Phe Leu Thr Leu Ser Ile Val
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Val Glu Ile Leu Phe Val Ile Ser Thr Phe Ala Val Gly Phe Glu Leu
            20                  25                  30

Phe Val Gly Ala Ala Val Val Ala Gly Gly Phe Phe Leu Val Ser Glu
        35                  40                  45

Val Leu Met Ile Val Ser Leu His Phe Tyr Met Pro Thr Thr Thr Thr
    50                  55                  60

Thr Val Thr Thr Thr Gly Leu Ala Val Met Glu Glu Lys Val Glu Glu
65                  70                  75                  80

Val Glu Glu Met Met Val Gly Lys Glu Gly Val Gly Glu Glu Asp Glu
                85                  90                  95

Glu Met Val Glu Glu Lys Val Asp Val Thr Thr Ala Ala Thr Thr Asn
            100                 105                 110

Ala Leu Leu Arg Thr Glu Lys Gln Arg Leu Leu Leu Ala Lys Glu Ser
        115                 120                 125

Ala Thr Thr Thr Thr Thr Thr Ala Thr Val Thr Thr Gly Gln Thr Ser
    130                 135                 140

Lys Thr Ser Thr Ser Phe Met Pro Val Arg Val Asp Glu Ala Ser Leu
145                 150                 155                 160

Glu Gln Phe Arg Arg Leu Thr Val Ile Thr Val Leu Ser Asn Met Gln
                165                 170                 175

Tyr Leu Pro Phe Leu Leu Pro Ile Leu Pro Phe Val Leu Ser Gly Leu
            180                 185                 190

Pro Leu Pro Val Ala Ser Phe His Trp Phe Gly Ala Phe Cys Cys Leu
        195                 200                 205

Thr Ser Ala Val Val Leu Asn Ala Tyr Val Lys Thr Thr Leu Ala Lys
    210                 215                 220

Ala Gly Asn Arg Ile Ser Ser Phe Gln Arg Ser Leu Leu Asn Val Leu
225                 230                 235                 240

Pro Thr Leu Ile Tyr Ala Ala Pro Gly Leu Ile Cys Phe Phe Ala Trp
                245                 250                 255
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Ser Gln His Gln Gly Gly Arg Glu Asp Gly Lys Glu Arg Ala Val Thr
            260                 265                 270

Ala Phe Pro Ala Trp Ala Ala Leu Thr Ala Met His Tyr Leu Tyr Leu
        275                 280                 285

Phe Leu Thr Phe Arg Gly Asn Pro Glu Val Thr Gly Glu Arg Tyr Leu
    290                 295                 300

Gly Glu Lys Leu Glu Leu Trp Lys Gly Gly Trp Ser Leu Tyr Tyr Phe
305                 310                 315                 320

Leu Glu Gly Ile Asp Gln Tyr Phe Gln Ala Lys Leu Val Phe Met Asp
                325                 330                 335

Pro Lys Leu Asp Leu Lys Gly Lys Pro His Val Phe Ala Phe His Pro
            340                 345                 350

His Gly Val Gln Pro Phe Thr Thr Phe Trp Ile Gln Leu Ser Arg Ala
        355                 360                 365

Trp Arg Glu Gly Val Gly Lys Gly Gln Arg Phe Cys Val Met Thr Ala
    370                 375                 380

Ser Val Met His Tyr Val Pro Leu Met Arg Asp Ile Leu Gln Trp Leu
385                 390                 395                 400

Gly Gly Arg Glu Val Ser Arg Glu Ala Ile Ser Tyr Ala Leu Asp Arg
                405                 410                 415

Lys Gln Ser Val Leu Leu Val Pro Gly Gly Gln Gln Glu Met Met Glu
            420                 425                 430

Ser Gln Ser Gln Met Gly Glu Ile Arg Ile Ile Thr Lys His Val Gly
        435                 440                 445

Phe Ile Arg Leu Ala Leu Gln Thr Gly Ala Pro Leu Val Pro Val Leu
    450                 455                 460

Ser Phe Gly Glu Val Glu Val Met Asp Phe Val Arg Tyr Pro Arg Leu
465                 470                 475                 480

Gln Arg Phe Phe Ile Ser Arg Ile Gly Ile Pro Val Pro Phe Phe Pro
                485                 490                 495

Tyr Gly Leu Phe Gly Phe Pro Ile Pro Arg Pro Val Pro Val Thr Val
            500                 505                 510

Val Phe Gly Arg Pro Ile Ala Val Glu Lys Val Glu Gln Pro Thr Gln
        515                 520                 525
```

Glu Glu Val Arg Lys Leu Ser Lys Lys Tyr Phe Glu Ser Ile Gln Glu
530 535 540

Val Phe Asp Lys Asn Lys Ala Lys Ala Leu Gly His Gly Asn His Lys
545 550 555 560

Leu Val Leu Leu

<210> 39
<211> 2074
<212> DNA
<213> Nannochloropsis oculata

<400> 39
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tgtcgattgt ggtagaaatc ttgtttgtga tctcgacgtt tgctgtgggg tttgagttgt 180

ttgttggagc ggcggtggtg gcgggcggct tctttttggt ctcggaagtg ttgatgattg 240

tgagtttgca tttttatatg cctacgacga ccacgactgt gacaacgacc gggttggcgg 300

tgatggagga gaaggtggag gaggtggagg agatgatggt ggggaaggag ggagtggggg 360

aagaggacga ggagatggtg gaggaaaagg tggacgtgac gacagcggcg acgacgaacg 420

cactcttaag aaccgaaaag cagcggctgc tcttggcgaa agagagtgct acgaccacta 480

ctactaccgc gactgtgacc acggggcaga ccagcaagac gtctacttca tttatgcctg 540

tccgggtcga cgaggcttcc cttgagcaat tccgccggct caccgttata accgttctga 600

gtaatatgca atacctgccc ttcctccttc ccatcctccc ttttgtcctc tcaggtcttc 660

ctctccctgt ggcatctttt cactggttcg gcgctttttg ttgtctgacc tcagcggtcg 720

ttttaaacgc ctatgtcaaa accacgttgg ccaaagctgg gaatcgtatt tcctccttcc 780

agcgctccct ccttaatgtc ctccccacgc tcatttatgc cgcgccgggt cttatttgct 840

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cgttcccggc ttgggcggcg ctcacggcca tgcattacct gtacctcttt ctcacgtttc 960

gcggaaatcc ggaagtaacg ggagagaggt acttaggcga aaagctagag ctgtggaaag 1020

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tcttcatgga cccgaaactg gatctgaagg ggaaaccgca tgtgtttgcg tttcacccac 1140

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tggggaaggg acagagattc tgtgtgatga ctgcgagtgt tatgcattat gtgccgttaa 1260

tgcgcgatat attacagtgg ctcgggggggc gggaagtgag cagggaagcc atttcgtacg 1320

cactggaccg taaacagtca gtattgttgg ttccaggcgg acaacaagag atgatggagt 1380

cccaatctca gatgggcgag attcggatca ttacgaagca cgtcggcttc attagattag 1440

cactccagac aggcgcgccg ctcgtgcctg tgctctcatt tggcgaagtt gaagtgatgg 1500

attttgtccg gtacccgcgt ctacagcgtt tctttatctc gcgcatcggt attccggttc 1560

ccttcttccc atatggattg tttggatttc ccatcccaag gcccgtgccc gtgacggtcg 1620

tgtttggccg tccgattgca gtggagaaag tggagcaacc gacgcaggaa gaggtgcgta 1680

aattgtcgaa aaagtacttt gaaagtatcc aggaggtgtt tgataaaaat aaggcgaagg 1740

ccctggggca tggaaatcat aaattggtcc tgttgtgagg gaggaagaga agcaaaaggg 1800

tgggagacag ggagatggat ggggagaagg aggtttgtgg gggtaggctt tcggagagag 1860

aacaaacgga ctgatacaag acaaaagtgt aagatagaac ttcaggaaag cgaaataatg 1920

attgaacgac atagaaaaaa gaaagggcag cgaggaaggg agggagggag gaagggagga 1980

cagtactgaa atgccaccaa tggcggtccc agcatcggag aatgcacaat aaagcaacaa 2040

agctagtcgg taatgaaaaa aaaaaaaaaa aaaa 2074

<210> 40
<211> 1029
<212> DNA
<213> Nannochloropsis oculata

<400> 40
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gggaagctgc ctatcggggc aatcaggctc ttcacgtccc ggcctgcttc atggcgtacc 120

actcccatgg tcgtgggcgg ctccttgctg gtggtgggat ccttcgtctg ggtgcccctt 180

gttatctggc tgggttggaa gaaatgtagg acacggaatc gacgcattgt ctacgtcctt 240

gttttgtgtg tcatcttgac cctacctaca cggcgttggg acgcggtggt cttgaacggc 300

ctatggagcc gttttgtgga atatttttca gtccaggtgg taggggacga ccccttgccc 360

aaggaccgct ccgccgtcta cgccgtcatt cctcacggca ccttcccctt tggtctcggc 420

gtggtctccc tcggtccctt gaacaagatc ttcaataagg tccggcccgt ggtggcctcg 480

gcagtcttgc gctttccggg ctttggtcaa ctaataggct tcgccggtgg ggtcgacgca 540

gggcccaaag aagtaagcaa ggccatcaag aagggctgtt cagtgagtat ctgtcctggg 600

ggcatcgcag agatgttctg gggatttcca aaggagggct gcttaccgcg ggaggaatat 660

gcgttcttac agtcgaggaa agggtttatc cgcatggcca tgaaacacaa tgtgcctgtg 720

gtccctgtgt actgttttgg taacacccac gcgatgcata aggcgaagac gccttgggtc 780

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cacgcagaaa atccaacccc tgctcagatt gaggcggcgc acgccgagtt ctgcagggcc 960

ctttcggatt tgtttgatcg gtacaagttt tattatggat gggggcacaa gacgcttcgc 1020

atcgtctga 1029

<210> 41
<211> 342
<212> PRT
<213> Nannochloropsis oculata

<400> 41

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Met Leu Met Ala Pro Ser Arg Arg Pro Ala Ser Ser Leu Val Asp Pro
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Leu Pro Leu Thr Gly Lys Leu Pro Ile Gly Ala Ile Arg Leu Phe Thr
            20                  25                  30

Ser Arg Pro Ala Ser Trp Arg Thr Thr Pro Met Val Val Gly Gly Ser
        35                  40                  45

Leu Leu Val Val Gly Ser Phe Val Trp Val Pro Leu Val Ile Trp Leu
    50                  55                  60

Gly Trp Lys Lys Cys Arg Thr Arg Asn Arg Arg Ile Val Tyr Val Leu
65                  70                  75                  80

Val Leu Cys Val Ile Leu Thr Leu Pro Thr Arg Arg Trp Asp Ala Val
                85                  90                  95

Val Leu Asn Gly Leu Trp Ser Arg Phe Val Glu Tyr Phe Ser Val Gln
            100                 105                 110

Val Val Gly Asp Asp Pro Leu Pro Lys Asp Arg Ser Ala Val Tyr Ala
        115                 120                 125

Val Ile Pro His Gly Thr Phe Pro Phe Gly Leu Gly Val Val Ser Leu
    130                 135                 140

Gly Pro Leu Asn Lys Ile Phe Asn Lys Val Arg Pro Val Val Ala Ser
145                 150                 155                 160

Ala Val Leu Arg Phe Pro Gly Phe Gly Gln Leu Ile Gly Phe Ala Gly
                165                 170                 175

Gly Val Asp Ala Gly Pro Lys Glu Val Ser Lys Ala Ile Lys Lys Gly
            180                 185                 190

Cys Ser Val Ser Ile Cys Pro Gly Gly Ile Ala Glu Met Phe Trp Gly
        195                 200                 205

Phe Pro Lys Glu Gly Cys Leu Pro Arg Glu Glu Tyr Ala Phe Leu Gln
    210                 215                 220

Ser Arg Lys Gly Phe Ile Arg Met Ala Met Lys His Asn Val Pro Val
225                 230                 235                 240

Val Pro Val Tyr Cys Phe Gly Asn Thr His Ala Met His Lys Ala Lys
                245                 250                 255

Thr Pro Trp Val Leu Glu Ala Leu Ser Arg Leu Leu Lys Thr Ser Leu
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260 265 270

Ile Leu Thr Trp Gly Arg Trp Gly Leu Pro Ile Pro Tyr Arg Val Pro
275 280 285

Leu Leu Tyr Ala Val Gly Lys Pro Leu Arg Leu Leu His Ala Glu Asn
290 295 300

Pro Thr Pro Ala Gln Ile Glu Ala Ala His Ala Glu Phe Cys Arg Ala
305 310 315 320

Leu Ser Asp Leu Phe Asp Arg Tyr Lys Phe Tyr Tyr Gly Trp Gly His
325 330 335

Lys Thr Leu Arg Ile Val
340

<210> 42
<211> 1585
<212> DNA
<213> Nannochloropsis oculata

<400> 42
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ggacccttttg ccattgacgg ggaagctgcc tatcggggca atcaggctct tcacgtcccg 180

gcctgcttca tggcgtacca ctcccatggt cgtgggcggc tccttgctgg tggtgggatc 240

cttcgtctgg gtgccccttg ttatctggct gggttggaag aaatgtagga cacggaatcg 300

acgcattgtc tacgtccttg ttttgtgtgt catcttgacc ctacctacac ggcgttggga 360

cgcggtggtc ttgaacggcc tatggagccg ttttgtggaa tatttttcag tccaggtggt 420

aggggacgac cccttgccca aggaccgctc cgccgtctac gccgtcattc ctcacggcac 480

cttccccttt ggtctcggcg tggtctccct cggtcccttg aacaagatct tcaataaggt 540

ccggcccgtg gtggcctcgg cagtcttgcg ctttccgggc tttggtcaac taataggctt 600

cgccggtggg gtcgacgcag ggcccaaaga agtaagcaag gccatcaaga agggctgttc 660

agtgagtatc tgtcctgggg gcatcgcaga gatgttctgg ggatttccaa aggagggctg 720

cttaccgcgg gaggaatatg cgttcttaca gtcgaggaaa gggtttatcc gcatggccat 780

gaaacacaat gtgcctgtgg tccctgtgta ctgttttggt aacacccacg cgatgcataa 840

ggcgaagacg ccttgggtct tggaggcgct atcaaggtca gtcacggggg aatagtgggg 900

ttgagtggga gacggcgggg gaaaatatat cttgattttt attgtaccgc atctgcgagg 960

ctgtctctaa tcgctttcta cgcgagacca ttcaaaattt tcgctatttc tttgcgtcgt 1020

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cacgcagaaa atccaacccc tgctcagatt gaggcggcgc acgccgagtt ctgcagggcc 1200

ctttcggatt tgtttgatcg gtacaagttt tattatggat gggggcacaa gacgcttcgc 1260

atcgtctgag aacgggggga ggggggggagg ggtcgttagg ttatgctgga aggaaagaga 1320

atgggagaga gggagagaga aagagtgggg aagatattga tggtatagtc ctcgtctggg 1380

aggcaattgc tgcttgggga ggctcccgag ggagaatgag ggagcgaaga gtagggaaac 1440

caaattatta aatctttttc cttcgttaag acttaggaat aaatgtaaag tacaaagaag 1500

aagagcccgt ctcttgcatc aaattgaaag aaataaagat aaccaatgaa ctaaaaaaaa 1560

aaaaaaaaaa aaaaaaaaaa aaaaa 1585

<210> 43
<211> 1251
<212> DNA
<213> Nannochloropsis oculata

<400> 43
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cgtaacgagg atatagtgcc ggaagcagcg acgggagacg gagcagcagg cgatgcaact 120

gctggtggcc tttctcgctc aacaccaaca gcggctccgg aggcctccac ttcgctttca 180

tcgcgactgg taccatcccc agcacaagtt tcatccatgc ccccagcaca agcttcagcc 240

acgcctattg tggtgcggcc cgaggcacgc cccgcaggtc cacaaggccg tctacaagca 300

ttaggtgcgg tgctattttt ggggctcatg gggtcgtcgc tgtacctagt gatcgcgtca 360

gcgctttaca tcgtgattgg tttcggtgtg ttgggccacc gcatttgccc ttcgatctta 420

ctcggggttt gggtaggaca agccctaatt tccgtcaagg tgctgcacca agacccggaa 480

ggtatcaagc ggtcgtggct tttccgagaa atggtgaact tttttgatgt gacactggtg 540

atggagcaga aattggacac ttccaagaag tacctatttg cacaacaccc gcacggtatc 600

cttcccctcg cccccgtgtt gtccgcttac tttgtctcgg acgtggtgcc cggcggaggc 660

aagatctttt gtttgataca tagcggcatc tttcacctgc ccatcgtccg ttttttcatg 720

ggtgaatggg gtgcactctc cgcaaacaag gagtctgtcg ccgaagcaaa gcaacaagga 780

cagcattgct ccatcgtcgt cggcggggtc gcggagattt tcctccaaaa cggagagacc 840

gagcaactgc aactcagaaa gggcttcatt cgtgaggcac ttcgtaatgg atatgacctt 900

gtgcccatgt ttcactttgg ggccacgcgc atgtatcatt ttgttggccc tgtttcattt 960

tggcggtcct tgtccaatta cctgccgttt ccctttttcc tcattggggg atggggaaaa 1020

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gtggatttga tatatgagga gtggaagaag cacttggcgg gcctgtatta tcggcagcgg 1200

cctgagtggg aaacgcggga gttggagatt ttggactgtc cgaagtcgtg a 1251

<210> 44
<211> 416
<212> PRT

<213> Nannochloropsis oculata

<400> 44

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Met Gly Ala Thr Thr Ala Thr Gln Thr Lys Lys Thr Leu Val Met Arg
1               5                  10                  15

Thr Val Ala Val Arg Asn Glu Asp Ile Val Pro Glu Ala Ala Thr Gly
            20                  25                  30

Asp Gly Ala Ala Gly Asp Ala Thr Ala Gly Gly Leu Ser Arg Ser Thr
        35                  40                  45

Pro Thr Ala Ala Pro Glu Ala Ser Thr Ser Leu Ser Ser Arg Leu Val
    50                  55                  60

Pro Ser Pro Ala Gln Val Ser Ser Met Pro Pro Ala Gln Ala Ser Ala
65                  70                  75                  80

Thr Pro Ile Val Val Arg Pro Glu Ala Arg Pro Ala Gly Pro Gln Gly
                85                  90                  95

Arg Leu Gln Ala Leu Gly Ala Val Leu Phe Leu Gly Leu Met Gly Ser
            100                 105                 110

Ser Leu Tyr Leu Val Ile Ala Ser Ala Leu Tyr Ile Val Ile Gly Phe
        115                 120                 125

Gly Val Leu Gly His Arg Ile Cys Pro Ser Ile Leu Leu Gly Val Trp
    130                 135                 140

Val Gly Gln Ala Leu Ile Ser Val Lys Val Leu His Gln Asp Pro Glu
145                 150                 155                 160

Gly Ile Lys Arg Ser Trp Leu Phe Arg Glu Met Val Asn Phe Phe Asp
                165                 170                 175

Val Thr Leu Val Met Glu Gln Lys Leu Asp Thr Ser Lys Lys Tyr Leu
            180                 185                 190

Phe Ala Gln His Pro His Gly Ile Leu Pro Leu Ala Pro Val Leu Ser
        195                 200                 205

Ala Tyr Phe Val Ser Asp Val Val Pro Gly Gly Gly Lys Ile Phe Cys
    210                 215                 220

Leu Ile His Ser Gly Ile Phe His Leu Pro Ile Val Arg Phe Phe Met
225                 230                 235                 240

Gly Glu Trp Gly Ala Leu Ser Ala Asn Lys Glu Ser Val Ala Glu Ala
                245                 250                 255
```

```
Lys Gln Gln Gly Gln His Cys Ser Ile Val Val Gly Gly Val Ala Glu
            260                 265                 270

Ile Phe Leu Gln Asn Gly Glu Thr Glu Gln Leu Gln Leu Arg Lys Gly
        275                 280                 285

Phe Ile Arg Glu Ala Leu Arg Asn Gly Tyr Asp Leu Val Pro Met Phe
    290                 295                 300

His Phe Gly Ala Thr Arg Met Tyr His Phe Val Gly Pro Val Ser Phe
305                 310                 315                 320

Trp Arg Ser Leu Ser Asn Tyr Leu Pro Phe Pro Phe Phe Leu Ile Gly
                325                 330                 335

Gly Trp Gly Lys Gly Leu Thr Leu Leu Pro Lys Pro Val Arg Ile Val
            340                 345                 350

Ile Ala Val Gly Ser Pro Ile Gly Leu Ala Ala Leu Tyr Gly Val Pro
        355                 360                 365

Glu Gly Gln Ser Val Pro Asp Pro Asp Leu Ala Lys Val Asp Leu Ile
    370                 375                 380

Tyr Glu Glu Trp Lys Lys His Leu Ala Gly Leu Tyr Tyr Arg Gln Arg
385                 390                 395                 400

Pro Glu Trp Glu Thr Arg Glu Leu Glu Ile Leu Asp Cys Pro Lys Ser
                405                 410                 415
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<210> 45
<211> 1923
<212> DNA
<213> Nannochloropsis oculata

<400> 45

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gaaagccacg ctgccacgct tgcataagaa caaaggggg catcaccacg cgacgctggg       120

gacggagaag gacatcaaac aaggacacaa gcatgggcgc taccactgcg acccagacta       180

aaaagacgtt ggtcatgcgg acagtcgcag tgcgtaacga ggatatagtg ccggaagcag       240

cgacgggaga cggagcagca ggcgatgcaa ctgctggtgg cctttctcgc tcaacaccaa       300

cagcggctcc ggaggcctcc acttcgcttt catcgcgact ggtaccatcc ccagcacaag       360

tttcatccat gcccccagca caagcttcag ccacgcctat tgtggtgcgg cccgaggcac       420

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tggggtcgtc gctgtaccta gtgatcgcgt cagcgcttta catcgtgatt ggtttcggtg       540

tgttgggcca ccgcatttgc ccttcgatct tactcggggt ttgggtagga caagccctaa       600

tttccgtcaa ggtgctgcac caagacccgg aaggtatcaa gcggtcgtgg cttttccgag       660
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aaatggtgaa ctttttgat gtgacactgg tgatggagca gaaattggac acttccaaga 720

agtacctatt tgcacaacac ccgcacggta tccttcccct cgcccccgtg ttgtccgctt 780

actttgtctc ggacgtggtg cccggcggag gcaagatctt ttgtttgata catagcggca 840

tctttcacct gcccatcgtc cgtttttca tgggtgaatg gggtgcactc tccgcaaaca 900

aggagtctgt cgccgaagca aagcaacaag gacagcattg ctccatcgtc gtcggcgggg 960

tcgcggagat tttcctccaa aacggagaga ccgagcaact gcaactcaga aagggcttca 1020

ttcgtgaggc acttcgtaat ggatatgacc ttgtgcccat gtttcacttt ggggccacgc 1080

gcatgtatca ttttgttggc cctgtttcat tttggcggtc cttgtccaat tacctgccgt 1140

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gtattgtaat tgctgtcggt tcgcccatag gccttgcggc tttgtatggg gtgccggaag 1260

gacagtcggt gcctgatcca gacctggcga aagtggattt gatatatgag gagtggaaga 1320

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ttttggactg tccgaagtcg tgagtgatta aaaagagatc gcatctgtgc gacgaagtgc 1440

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cagagtagga ggatttgaca aagattaaga cgtggacgac atgacgacat gggagaaaga 1560

ctggtcgaat ttaaccaaaa aaagagctac cgcagcaagc gtaacgcaga ggagcattta 1620

agtatgcatg ttgccaaggc aaggcaaggc aaaaggccat ccgagtagca ggcacacgca 1680

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ctgtcatcaa cataagtaag atacgaaaga cacagaagga taagtgggag gatggggtgt 1860

agtaggaggg tggggaggtt ggatggaaaa ggggggttcg gcgagtggag ttggacaggg 1920

ccc 1923

<210> 46
<211> 930
<212> DNA
<213> Thraustochytrium aureum

<400> 46
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acttatgtgg tgctgacggc tgtgctggcc ctgcacccga tcccggacat ctcggatgcc 180

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ctgcgctact ttaccatgtt caagtcggtc acggtgtcac gcgagagcct caccaaacag 480

ctggagctcg ggaacacggt tggcctggtt ggcgatggca tcgctgggat cttccaatgc 540

gaccacaacg acgaggtcgt tgcgctccgg acgcgcaagg ggctcgcaaa actggcgctg 600

cgaacggggc ggcccgtttt gccctgctac agcttgggaa acacggaagc gtttagcgtt 660

tggtttgacc gctggggcgt catggagcgc ctctcgcgca agctgcaggc gagcgtgttt 720

ttctactggg gcaggtacgg cctccctgtt ccgtaccgtg tcaatatcac gatgatcctc 780

ggcgacatgg tcctcgtcga ccaggtcgag aacccgacgc cggcacaggt cgatgcagtg 840

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tggggccaca agacgatgcg atttgtgtag 930

<210> 47
<211> 309
<212> PRT
<213> Thraustochytrium aureum

<400> 47

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20 25 30

Trp Gly Ser Trp Ser Gln Val Ala Thr Tyr Val Val Leu Thr Ala Val
35 40 45

Leu Ala Leu His Pro Ile Pro Asp Ile Ser Asp Ala Val Tyr Ser Ser
50 55 60

Trp Ile Val Gln Gln Leu Tyr Lys Tyr Phe Thr Tyr Arg Phe Val Tyr
65 70 75 80

Ser Gly Asn Ala Arg Val Leu Ala Gln Thr Gln Ala Pro Phe Ile Gly
85 90 95

Ala Gly Val Pro His Gly Ala Met Pro Phe Ser Asn Leu Leu Ser Val
100 105 110

Pro Ala Val Asn Ser Phe Ser Pro Ser Gln Thr Gly Gly Glu Phe Val
115 120 125

Gly Ala Pro Ala Ser Ile Val Phe Arg Thr Pro Phe Leu Arg Tyr Phe
130 135 140

Thr Met Phe Lys Ser Val Thr Val Ser Arg Glu Ser Leu Thr Lys Gln
145 150 155 160

Leu Glu Leu Gly Asn Thr Val Gly Leu Val Gly Asp Gly Ile Ala Gly
165 170 175

Ile Phe Gln Cys Asp His Asn Asp Glu Val Val Ala Leu Arg Thr Arg

180 185 190

Lys Gly Leu Ala Lys Leu Ala Leu Arg Thr Gly Arg Pro Val Leu Pro
195 200 205

Cys Tyr Ser Leu Gly Asn Thr Glu Ala Phe Ser Val Trp Phe Asp Arg
210 215 220

Trp Gly Val Met Glu Arg Leu Ser Arg Lys Leu Gln Ala Ser Val Phe
225 230 235 240

Phe Tyr Trp Gly Arg Tyr Gly Leu Pro Val Pro Tyr Arg Val Asn Ile
245 250 255

Thr Met Ile Leu Gly Asp Met Val Leu Val Asp Gln Val Glu Asn Pro
260 265 270

Thr Pro Ala Gln Val Asp Ala Val His Glu Arg Ile Leu Ala Ser Ile
275 280 285

Glu Asn Ala Phe Asn Arg His Lys Ala Ala Leu Gly Trp Gly His Lys
290 295 300

Thr Met Arg Phe Val
305

<210> 48
<211> 1134
<212> DNA
<213> Thraustochytrium aureum

<400> 48
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gtgtaggagg tgctgtttgc caacaccaca cttggcctgg cctgggatgc ggctgggcca 1020

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gccataaaga gtcgaacgaa aatagcaaaa tgtgcaattc accaaaaaaa aaaa 1134

<210> 49
<211> 1179
<212> DNA
<213> Thraustochytrium aureum

<400> 49
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gccgtgtggt ggacgctctt gtgggcgatt gcgttttgga cgatctttta cgccgcgctc 180

aagaattggg gcgtgcgagg gtggcggctc agcctggcgc tcgctgtctt cgcggtctgc 240

tcgttcggcg gcactctgcg gtaccactcg gagagcccac actacccgat ggcggttctc 300

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ttcttcggcc tgcaggtcct gctcaccgac ggtgccaagc gcgtcgcgca catgctgggc 480

gacgagtcga gcgcagaccc gcggatgcgc caggtaatgc tcctcttcca cccgcacagc 540

atcttcccag tctcgcacgc ggcgctgggt ctcacttcgc tctggcgctc gcactttccc 600

cacctctcgg tcaacccccc aacagcgagc attatccact ttgtgccggt catgcgcgac 660

gttttgcagt ggctcggcat ctgcgacgtc tccaaagcga gcgtggtcaa cctcatcggc 720

atggggcgca acgtccagat cgtgtgcggc gggcagaccg agatgttcga gtcccgctcc 780

tgggacaagg agatttctgt ggtgcgggcg cgccgccttg gcgttttcaa gatcgccatc 840

cagcagggcc tcggtatcgt gccgatttac agcttcggag agccgctcac ctttgacaac 900

atatacatgc cccgcttgca aaacttttgc aagcgcgtgc tcggcttccc ctgcccgttc 960

gtgatgctcg gtcagtacgg ccttcccatt ccgcgccgcg tcccaatttc ggtggctgtt 1020

ggcgagcccg tctttcctgc tcggcagacc gccgatcctt cgctcgagga ggtcaaagag 1080

tttcacagac gttactttga ggccctgcag gccctgtttg accagttcaa ggaccaggcc 1140

gggcacggcc agtgtagcat caagtggctg gactcgtag 1179

<210> 50
<211> 392
<212> PRT
<213> Thraustochytrium aureum

<400> 50

Met Val Phe Leu Cys Leu Pro Tyr Met Leu Pro Glu Ala Leu Leu Pro
1 5 10 15

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Phe Leu Asp Thr Ala Thr Leu Gly Leu Ile Pro Ala Leu Pro Gly Asp
            20                  25                  30

Lys Glu Asn Phe Val His Thr Phe Ala Val Trp Trp Thr Leu Leu Trp
        35                  40                  45

Ala Ile Ala Phe Trp Thr Ile Phe Tyr Ala Ala Leu Lys Asn Trp Gly
    50                  55                  60

Val Arg Gly Trp Arg Leu Ser Leu Ala Leu Ala Val Phe Ala Val Cys
65                  70                  75                  80

Ser Phe Gly Gly Thr Leu Arg Tyr His Ser Glu Ser Pro His Tyr Pro
                85                  90                  95

Met Ala Val Leu Ile Cys Ser Leu Asn Phe Val Tyr Ile Ser Thr Thr
            100                 105                 110

Phe Thr Lys Lys Pro Glu Ser Asn Ala Cys Arg Glu Trp Pro Glu Leu
        115                 120                 125

Arg Glu Leu Arg Ile Leu Pro Asp Met Phe Glu Arg Phe Phe Gly Leu
    130                 135                 140

Gln Val Leu Leu Thr Asp Gly Ala Lys Arg Val Ala His Met Leu Gly
145                 150                 155                 160

Asp Glu Ser Ser Ala Asp Pro Arg Met Arg Gln Val Met Leu Leu Phe
                165                 170                 175

His Pro His Ser Ile Phe Pro Val Ser His Ala Ala Leu Gly Leu Thr
            180                 185                 190

Ser Leu Trp Arg Ser His Phe Pro His Leu Ser Val Asn Pro Leu Thr
        195                 200                 205

Ala Ser Ile Ile His Phe Val Pro Val Met Arg Asp Val Leu Gln Trp
    210                 215                 220

Leu Gly Ile Cys Asp Val Ser Lys Ala Ser Val Val Asn Leu Ile Gly
225                 230                 235                 240

Met Gly Arg Asn Val Gln Ile Val Cys Gly Gly Gln Thr Glu Met Phe
                245                 250                 255

Glu Ser Arg Ser Trp Asp Lys Glu Ile Ser Val Val Arg Ala Arg Arg
            260                 265                 270

Leu Gly Val Phe Lys Ile Ala Ile Gln Gln Gly Leu Gly Ile Val Pro
        275                 280                 285
```

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Ile Tyr Ser Phe Gly Glu Pro Leu Thr Phe Asp Asn Ile Tyr Met Pro
    290                 295                 300

Arg Leu Gln Asn Phe Cys Lys Arg Val Leu Gly Phe Pro Cys Pro Phe
305                 310                 315                 320

Val Met Leu Gly Gln Tyr Gly Leu Pro Ile Pro Arg Arg Val Pro Ile
                325                 330                 335

Ser Val Ala Val Gly Glu Pro Val Phe Pro Ala Arg Gln Thr Ala Asp
            340                 345                 350

Pro Ser Leu Glu Glu Val Lys Glu Phe His Arg Arg Tyr Phe Glu Ala
        355                 360                 365

Leu Gln Ala Leu Phe Asp Gln Phe Lys Asp Gln Ala Gly His Gly Gln
    370                 375                 380

Cys Ser Ile Lys Trp Leu Asp Ser
385                 390
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<210> 51
<211> 1303
<212> DNA
<213> Thraustochytrium aureum

<400> 51

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ttttacgccg cgctcaagaa ttggggcgtg cgagggtggc ggctcagcct ggcgctcgct      240

gtcttcgcgg tctgctcgtt cggcggcact ctgcggtacc actcggagag cccacactac      300

ccgatggcgg ttctcatctg ctcgctcaac tttgtctaca tctccactac gttcaccaag      360

aagccagagt ccaacgcgtg ccgggagtgg cccgagctgc gcgagctgcg catcttgccc      420

gacatgtttg agcgcttctt cggcctgcag gtcctgctca ccgacggtgc caagcgcgtc      480

gcgcacatgc tgggcgacga gtcgagcgca gacccgcgga tgcgccaggt aatgctcctc      540

ttccacccgc acagcatctt cccagtctcg cacgcggcgc tgggtctcac ttcgctctgg      600

cgctcgcact ttccccacct ctcggtcaac cccctaacag cgagcattat ccactttgtg      660

ccggtcatgc gcgacgtttt gcagtggctc ggcatctgcg acgtctccaa agcgagcgtg      720

gtcaacctca tcggcatggg gcgcaacgtc cagatcgtgt gcggcgggca gaccgagatg      780

ttcgagtccc gctcctggga caaggagatt tctgtggtgc gggcgcgccg ccttggcgtt      840

ttcaagatcg ccatccagca gggcctcggt atcgtgccga tttacagctt cggagagccg      900

ctcacctttg acaacatata catgccccgc ttgcaaaact tttgcaagcg cgtgctcggc      960
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ttcccctgcc cgttcgtgat gctcggtcag tacggccttc ccattccgcg ccgcgtccca 1020

atttcggtgg ctgttggcga gcccgtcttt cctgctcggc agaccgccga tccttcgctc 1080

gaggaggtca aagagtttca cagacgttac tttgaggccc tgcaggccct gtttgaccag 1140

ttcaaggacc aggccgggca cggccagtgt agcatcaagt ggctggactc gtagaggcag 1200

aaagccccgc gcactgcttt tgcgcctgtg ccgttcccgt ttgtagaaac aaccttccaa 1260

cattcgttag ctttctctta aaaaaaaaaa aaaaaaaaaa aaa 1303

<210> 52
<211> 1389
<212> DNA
<213> Thraustochytrium aureum

<400> 52
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ggcgttgtct ccacgatgat ctggaccccg atcctgatcg ggctccgctg cttcaacatc 120

tggctctccg tggttacctg gccgctctcg tttctggctc gcgtcgtttt cggcatggag 180

atgaagaagg cgagcttctg ggacgtccct ctggagcggc gcaagcagac ggtggcagtt 240

gcgggcttcg tgatgctgct cccctgcgtg ctgcttgcgt acgtctggtc gcttgtgctg 300

ctcgttttcc cgctgacgac gctgccaatg ctcgggtact acatctggat cttcaagatc 360

gacaagagcc ccgagaacgg gcagcgcacg ccgttcctgc gttactggtc ggcgtggcgc 420

cacttcgcct cctacttccc gctgcgcctc atcaagacgc acaacctcga cccgagccgc 480

aagtacgtct tcgcgtacca cccgcacggc atcatcagca ttggcgcgtt cggcaacttt 540

gccaccaacg cgacggggtt tagccgcaag tttcccggaa tcgacctccg cctcctcacc 600

ttggaaatga acttttggtg cccctggatc cgcgagttcc tgctgagcat gggcgtctgc 660

tcagccgcca agcggtcctg caacaagatt ctcagcaagg ggcccggaag cgccatcatg 720

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ctcgccttcg gggagaacga catctttgac accatctact acgagtccgg caccgtgatg 900

cgcaagatcc aggaggtcgt gcgcaagcgc ctcggctttg ccacccctgt ttttccggc 960

cgcggcttct tcaactacag ctttggcttc ctcccgcacc ggcgcccggt cattgtcgtc 1020

tgcgggcgcc ctatcaaggt cccaaaactc ccggaacacc tgcgcggctc ggcgctctcg 1080

accacccctg aaggcgtcgc gcttgtcgac cagtaccacc aaaagtacgt cgccgagctg 1140

cgccgcgtgt gggacctcta caagtccaag tgggccgtct cgcgggcaga gtcgctcatg 1200

atcaagggtg tgcaaaaccc ggcgctcccg cgctccccgt cgcgccgcat cccgccggcg 1260

cagcgcgttc ccgcgagtgc cgcctcgctt tcgtttcgcg aggtcgacga ggccgaattt 1320

gaggccaagg aggacggcgc gacctcttcg ccgcagtcca tgtctgcggc gctgtacacc 1380

gagggttag 1389

<210> 53
<211> 462
<212> PRT
<213> Thraustochytrium aureum

<400> 53

Met Phe Leu Arg Ile Glu Arg Glu Trp Arg Glu Glu Asp Glu Trp Ala
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Lys Gln Glu Pro Gly Val Val Ser Thr Met Ile Trp Thr Pro Ile Leu
20 25 30

Ile Gly Leu Arg Cys Phe Asn Ile Trp Leu Ser Val Val Thr Trp Pro
35 40 45

Leu Ser Phe Leu Ala Arg Val Val Phe Gly Met Glu Met Lys Lys Ala
50 55 60

Ser Phe Trp Asp Val Pro Leu Glu Arg Arg Lys Gln Thr Val Ala Val
65 70 75 80

Ala Gly Phe Val Met Leu Leu Pro Cys Val Leu Leu Ala Tyr Val Trp
85 90 95

Ser Leu Val Leu Leu Val Phe Pro Leu Thr Thr Leu Pro Met Leu Gly
100 105 110

Tyr Tyr Ile Trp Ile Phe Lys Ile Asp Lys Ser Pro Glu Asn Gly Gln
115 120 125

Arg Thr Pro Phe Leu Arg Tyr Trp Ser Ala Trp Arg His Phe Ala Ser
130 135 140

Tyr Phe Pro Leu Arg Leu Ile Lys Thr His Asn Leu Asp Pro Ser Arg
145 150 155 160

Lys Tyr Val Phe Ala Tyr His Pro His Gly Ile Ile Ser Ile Gly Ala
165 170 175

Phe Gly Asn Phe Ala Thr Asn Ala Thr Gly Phe Ser Arg Lys Phe Pro
180 185 190

Gly Ile Asp Leu Arg Leu Leu Thr Leu Glu Met Asn Phe Trp Cys Pro
195 200 205

Trp Ile Arg Glu Phe Leu Leu Ser Met Gly Val Cys Ser Ala Ala Lys
210 215 220

Arg Ser Cys Asn Lys Ile Leu Ser Lys Gly Pro Gly Ser Ala Ile Met
225 230 235 240

Leu Val Val Gly Gly Ala Ala Glu Ser Leu Asp Thr Glu Pro Gly Thr

245 250 255

Tyr Arg Leu Thr Leu Gly Arg Lys Gly Phe Ile Arg Val Ala Leu Asp
260 265 270

Asn Gly Ala Asp Leu Val Pro Val Leu Ala Phe Gly Glu Asn Asp Ile
275 280 285

Phe Asp Thr Ile Tyr Tyr Glu Ser Gly Thr Val Met Arg Lys Ile Gln
290 295 300

Glu Val Val Arg Lys Arg Leu Gly Phe Ala Thr Pro Val Phe Ser Gly
305 310 315 320

Arg Gly Phe Phe Asn Tyr Ser Phe Gly Phe Leu Pro His Arg Arg Pro
325 330 335

Val Ile Val Val Cys Gly Arg Pro Ile Lys Val Pro Lys Leu Pro Glu
340 345 350

His Leu Arg Gly Ser Ala Leu Ser Thr Thr Pro Glu Gly Val Ala Leu
355 360 365

Val Asp Gln Tyr His Gln Lys Tyr Val Ala Glu Leu Arg Arg Val Trp
370 375 380

Asp Leu Tyr Lys Ser Lys Trp Ala Val Ser Arg Ala Glu Ser Leu Met
385 390 395 400

Ile Lys Gly Val Gln Asn Pro Ala Leu Pro Arg Ser Pro Ser Arg Arg
405 410 415

Ile Pro Pro Ala Gln Arg Val Pro Ala Ser Ala Ala Ser Leu Ser Phe
420 425 430

Arg Glu Val Asp Glu Ala Glu Phe Glu Ala Lys Glu Asp Gly Ala Thr
435 440 445

Ser Ser Pro Gln Ser Met Ser Ala Ala Leu Tyr Thr Glu Gly
450 455 460

<210> 54
<211> 1547
<212> DNA
<213> Thraustochytrium aureum

<400> 54
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atcgggctcc gctgcttcaa catctggctc tccgtggtta cctggccgct ctcgtttctg 180

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cggcgcaagc agacggtggc agttgcgggc ttcgtgatgc tgctcccctg cgtgctgctt 300

gcgtacgtct ggtcgcttgt gctgctcgtt ttcccgctga cgacgctgcc aatgctcggg 360

tactacatct ggatcttcaa gatcgacaag agccccgaga acgggcagcg cacgccgttc 420

ctgcgttact ggtcggcgtg gcgccacttc gcctcctact tcccgctgcg cctcatcaag 480

acgcacaacc tcgacccgag ccgcaagtac gtcttcgcgt accacccgca cggcatcatc 540

agcattggcg cgttcggcaa ctttgccacc aacgcgacgg ggtttagccg caagtttccc 600

ggaatcgacc tccgcctcct caccttggaa atgaactttt ggtgccctg gatccgcgag 660

ttcctgctga gcatgggcgt ctgctcagcc gccaagcggt cctgcaacaa gattctcagc 720

aaggggcccg gaagcgccat catgctggtc gttggcggcg ccgccgagtc gctcgacacg 780

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ccgtcgcgcc gcatcccgcc ggcgcagcgc gttcccgcga gtgccgcctc gctttcgttt 1320

cgcgaggtcg acgaggccga atttgaggcc aaggaggacg gcgcgacctc ttcgccgcag 1380

tccatgtctg cggcgctgta caccgagggt tagtcctcat cagcttgccg atctcgccat 1440

cccgcccctg cctcgcgtcc cgccgagccg agttttgtca tgcaccagcg ccttcctgtt 1500

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<210> 55
<211> 1977
<212> DNA
<213> Nannochloropsis oculata

<400> 55
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gcctctctcg gcatcccctc tcgatggttc gcctacccct gcctggtcat gcttggccac 180

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ggccctgtca tcttctccgg gaaccactcg aaccaatttg tcgacgggat catggtcctc 360

accaccgccc aacaccgcgt cggcttcctt atcgccgaaa agtcctacaa ccaccctgtt 420

gtcggcacat ttgcaaaact cgcgggcgcc gtgcccgtca cccgccctca agacagcgct 480

aagctcatgc aaggtaccat tatcatgtcc ggccgctctg tcaagggaca aggaaccgcc 540

tttagtcacg agctcgtccc cggcgacaag ctacgtctaa aaggtggtgc tgatcaattc 600

aaagtcgagt ccatcacctc cgataccgag ctgatgctct ccgagaacgg ccccccttcct 660

ccccccctcct ctacctccgc ctcgcccttt gaaaaactag ggaaggtgga ccagacccgt 720

gtctacaatg ccgtgttcga gcaccttaag cacgggaaat gcatcggtat cttccccgaa 780

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ttccgaggcc accggtttcg tggacgggtg gtagtagaat tcgggccagc aattcgcgtg 960

ccggaagagt tggcggagtt gtacaagacc aatcgacgcg aggcgtatca ccagtttctg 1020

accaacgtgg aggaagggat gcgggcgacg cttgtgacgg cgcctgatta ccacgcgttg 1080

catttggtgt acacggcacg gaggttattt cagaaggata attggattcc gagcccacgg 1140

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cccgtcgtta tgcgtctttt acccggagct cgtaagaaaa tggcgaccct ccctgcggag 1740

cgcgcgcagc tacaaagaga aatccgcgcc tacatacacc agatcggccc tgaacttggg 1800

agtctctaca ccgacaaaac cgtcaagtgg gaagaatacg tccgcaagtc ctcatcggcg 1860

gctgacttgc aatcgttgtt gaacgaagcg acccaaccca agatgcaagg aagtcagacg 1920

gaaggaggga atggtggaga aaaaggggga aggaaggggg aagaggagct tgtctga 1977

<210> 56
<211> 658
<212> PRT
<213> Nannochloropsis oculata

<400> 56

Met Pro Ser Arg Ser Thr Ile Glu Val Ile Lys Ala Asp Lys Asn Gln
1 5 10 15

Asn Asn Leu Ala Tyr Gly Leu Ile Val Val Ile Leu Leu Ala Ile Asp
20 25 30

Pro Asn Pro Val Lys Val Ile Ala Ala Ser Leu Gly Ile Pro Ser Arg
35 40 45

```
Trp Phe Ala Tyr Pro Cys Leu Val Met Leu Gly His Leu Phe Leu Thr
    50                  55                  60

His Ser Gln Glu Phe Leu Tyr Asp Gly Val Arg Val Phe Phe Arg Ser
65                  70                  75                  80

Ile Leu Ser Ile Phe Phe Arg Gln Val Asp Ile Val Gly Ile Asp Asn
                85                  90                  95

Ile Pro Lys His Gly Pro Val Ile Phe Ser Gly Asn His Ser Asn Gln
            100                 105                 110

Phe Val Asp Gly Ile Met Val Leu Thr Thr Ala Gln His Arg Val Gly
        115                 120                 125

Phe Leu Ile Ala Glu Lys Ser Tyr Asn His Pro Val Val Gly Thr Phe
    130                 135                 140

Ala Lys Leu Ala Gly Ala Val Pro Val Thr Arg Pro Gln Asp Ser Ala
145                 150                 155                 160

Lys Leu Met Gln Gly Thr Ile Ile Met Ser Gly Arg Ser Val Lys Gly
                165                 170                 175

Gln Gly Thr Ala Phe Ser His Glu Leu Val Pro Gly Asp Lys Leu Arg
            180                 185                 190

Leu Lys Gly Gly Ala Asp Gln Phe Lys Val Glu Ser Ile Thr Ser Asp
        195                 200                 205

Thr Glu Leu Met Leu Ser Glu Asn Gly Pro Leu Pro Pro Pro Ser Ser
    210                 215                 220

Thr Ser Ala Ser Pro Phe Glu Lys Leu Gly Lys Val Asp Gln Thr Arg
225                 230                 235                 240

Val Tyr Asn Ala Val Phe Glu His Leu Lys His Gly Lys Cys Ile Gly
                245                 250                 255

Ile Phe Pro Glu Gly Gly Ser His Asp Arg Thr Asp Leu Leu Pro Leu
            260                 265                 270

Lys Val Gly Ile Ala Leu Ile Ala Cys Gly Met Val Asp Lys Tyr Asn
        275                 280                 285

Ile Thr Val Pro Ile Val Pro Val Gly Leu Asn Tyr Phe Arg Gly His
    290                 295                 300

Arg Phe Arg Gly Arg Val Val Val Glu Phe Gly Pro Ala Ile Arg Val
305                 310                 315                 320
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Pro Glu Glu Leu Ala Glu Leu Tyr Lys Thr Asn Arg Arg Glu Ala Tyr
                325                 330                 335

His Gln Phe Leu Thr Asn Val Glu Glu Gly Met Arg Ala Thr Leu Val
            340                 345                 350

Thr Ala Pro Asp Tyr His Ala Leu His Leu Val Tyr Thr Ala Arg Arg
        355                 360                 365

Leu Phe Gln Lys Asp Asn Trp Ile Pro Ser Pro Arg Glu Lys Met Asp
    370                 375                 380

Leu Asn Arg Arg Phe Ala Glu Gly Tyr Lys Ile Leu Met Asn Lys Tyr
385                 390                 395                 400

Gly Glu Gln Arg Pro Ala Ala Leu Val Glu Leu Glu Arg Arg Leu Asn
                405                 410                 415

Asp Tyr Gln Lys Thr Leu His Thr Leu Gly Leu Arg Asp Tyr Gln Val
            420                 425                 430

Pro Thr Leu Glu Glu Asp Asp Asn Leu Lys Leu Cys Tyr Thr Ile Ala
        435                 440                 445

His Leu Phe Leu Val Leu Thr Leu Ala Met Met Pro Ser Leu Val Leu
    450                 455                 460

Asn Ala Pro Val Gly Leu Ile Ala Arg Ile Val Ser Ser Arg Glu Gln
465                 470                 475                 480

Lys Lys Ala Leu Ala Ala Ser Arg Val Lys Ile Glu Ala Arg Asp Val
                485                 490                 495

Val Met Ser Lys Lys Ile Thr Leu Ser Ile Val Leu Val Pro Thr Leu
            500                 505                 510

Trp Ile Val Tyr Ala Ile Leu Leu Leu Arg Tyr Thr Ser Leu Gln Pro
        515                 520                 525

Ser Thr Val Ala Val Leu Phe Phe Ser Cys Pro Leu Phe Ser Tyr Leu
    530                 535                 540

Gly Val Met Ala Thr Glu Ala Gly Met Val Asp Ala Lys Asp Leu Lys
545                 550                 555                 560

Pro Val Val Met Arg Leu Leu Pro Gly Ala Arg Lys Lys Met Ala Thr
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Leu Pro Ala Glu Arg Ala Gln Leu Gln Arg Glu Ile Arg Ala Tyr Ile
            580                 585                 590
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His Gln Ile Gly Pro Glu Leu Gly Ser Leu Tyr Thr Asp Lys Thr Val
        595                 600                 605

Lys Trp Glu Glu Tyr Val Arg Lys Ser Ser Ser Ala Ala Asp Leu Gln
    610                 615                 620

Ser Leu Leu Asn Glu Ala Thr Gln Pro Lys Met Gln Gly Ser Gln Thr
625                 630                 635                 640

Glu Gly Gly Asn Gly Gly Glu Lys Gly Gly Arg Lys Gly Glu Glu Glu
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Leu Val
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<210> 57
<211> 2460
<212> DNA
<213> Nannochloropsis oculata

<400> 57

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aggaacaagc gcggccacca gggctcattt cgaatcaagc acatccgttc cacacccggg      180

caacaaaacc atgccatccc gcagcaccat tgaggtcatt aaggccgata agaaccagaa      240

taatctggcg tatggcctga ttgttgtcat cctcctggcc attgacccca accccgtcaa      300

agtcatcgcc gcctctctcg gcatcccctc tcgatggttc gcctacccct gcctggtcat      360

gcttggccac ctattcctca cccactccca ggaatttctc tacgacggcg tccgggtctt      420

cttccgctcc atcctttcga tcttcttccg tcaagtcgac attgtgggca tcgacaacat      480

cccgaaacac ggccctgtca tcttctccgg gaaccactcg aaccaatttg tcgacgggat      540

catggtcctc accaccgccc aacaccgcgt cggcttcctt atcgccgaaa agtcctacaa      600

ccaccctgtt gtcggcacat ttgcaaaact cgcgggcgcc gtgcccgtca cccgccctca      660

agacagcgct aagctcatgc aaggtaccat tatcatgtcc ggccgctctg tcaagggaca      720

aggaaccgcc tttagtcacg agctcgtccc cggcgacaag ctacgtctaa aaggtggtgc      780

tgatcaattc aaagtcgagt ccatcacctc cgataccgag ctgatgctct ccgagaacgg      840

ccccccttcct ccccccctcct ctacctccgc ctcgcccttt gaaaaactag ggaaggtgga      900

ccagacccgt gtctacaatg ccgtgttcga gcaccttaag cacgggaaat gcatcggtat      960

cttccccgaa ggcggctcgc acgatcggac agacctccta cccctcaagg tagggattgc     1020

actcatcgcc tgcggcatgg tcgataaata caatatcaca gtgcccatcg tccccgtggg     1080

tttgaactac ttccgaggcc accggtttcg tggacgggtg gtagtagaat tcgggccagc     1140

aattcgcgtg ccggaagagt tggcggagtt gtacaagacc aatcgacgcg aggcgtatca     1200
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gagcccacgg gagaagatgg atttgaaccg gcggtttgcc gaggggtata aaattttgat 1380

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tgaacttggg agtctctaca ccgacaaaac cgtcaagtgg gaagaatacg tccgcaagtc 2040

ctcatcggcg gctgacttgc aatcgttgtt gaacgaagcg acccaaccca agatgcaagg 2100

aagtcagacg gaaggaggga atggtggaga aaaaggggga aggaaggggg aagaggagct 2160

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<211> 104
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<213> Artificial

<220>
<223> Primer

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accccggatc ggcgcgccac catggacaag gcactggcac cgtt 104

<210> 59
<211> 101
<212> DNA
<213> Artificial

<220>
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<400> 59
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tagagcggat ttaattaact aaactttctt ccttccctct a 101

<210> 60
<211> 104
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 60
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accccggatc ggcgcgccac catgaccacg actgtcatct ctag 104

<210> 61
<211> 101
<212> DNA
<213> Artificial

<220>
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<400> 61
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tagagcggat ttaattaatc aaagcctccc gcacaacgag c 101

<210> 62
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<212> DNA
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<400> 62
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accccggatc ggcgcgccac catggagggc atcgagtcga tagt 104

<210> 63
<211> 101
<212> DNA
<213> Artificial

<220>
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<400> 63
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tagagcggat ttaattaact ataaggcttc tcccggcgcg g 101

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<400> 64

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accccggatc ggcgcgccac catgaagacg cccacgagcc tggc 104

<210> 65
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 65
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tagagcggat ttaattaatt aagctctcga atcgtccttc t 101

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<211> 104
<212> DNA
<213> Artificial

<220>
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<400> 66
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accccggatc ggcgcgccac catggtcagg aggaagatgg acgt 104

<210> 67
<211> 101
<212> DNA
<213> Artificial

<220>
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<400> 67
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tagagcggat ttaattaatc acgacgccgg cgccttgcag t 101

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accccggatc ggcgcgccac catggcaccc tccccaccgg cccc 104

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accccggatc ggcgcgccac catgggtcta tttggcagcg ggat 104

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accccggatc ggcgcgccac catgttgagt atccccgagt cgtc 104

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<220>
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<400> 73
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tagagcggat ttaattaact aaaagaaatc cagctccctg t 101

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accccggatc ggcgcgccac catgacgccg caagccgata tcac 104

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<400> 77
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<212> DNA
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accccggatc ggcgcgccac catgttgttg cagggattaa gctg 104

<210> 83
<211> 101
<212> DNA
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<400> 83
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<220>
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accccggatc ggcgcgccac catgttgatg gcgccgtcgc ggcg 104

<210> 85
<211> 101
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<220>
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<400> 85
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accccggatc ggcgcgccac catgggcgct accactgcga ccca 104

<210> 87
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 87
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tagagcggat ttaattaatc acgacttcgg acagtccaaa a 101

<210> 88
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<212> DNA
<213> Artificial

<220>
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accccggatc ggcgcgccac catgtcgttc gttgagcaca gcgc 104

<210> 89
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 89
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tagagcggat ttaattaact acacaaatcg catcgtcttg t 101

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accccggatc ggcgcgccac catggtcttc ctctgccttc ccta 104

<210> 91
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

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tagagcggat ttaattaact acgagtccag ccacttgatg c 101

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<212> DNA
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<220>
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<211> 104
<212> DNA
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<210> 95
<211> 101
<212> DNA
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<220>
<223> Primer

<400> 95
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<211> 1197
<212> DNA
<213> Phytophtora sojae

<400> 96
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<210> 97
<211> 1371
<212> DNA
<213> Ostreococcus tauri

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<210> 98
<211> 1320
<212> DNA
<213> Thraustochytrium sp.

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

<400> 99
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ctcgttgatt ctccaactcc aattgtgttg ggagtgtctg tttacttgac catcgtgatc 180

ggaggattgc tttggatcaa ggctagagat ctcaagccaa gagcttctga gccattcttg 240

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            260                 265                 270

Pro Val Val Leu Pro Arg Leu Glu Lys Pro Thr Glu Glu Glu Val Arg
        275                 280                 285

Lys Tyr His Ser Leu Tyr Val Arg Ala Leu Met Glu Leu Phe Glu Lys
    290                 295                 300

His Lys Thr Gln Tyr Cys Glu Lys Gly Ala Lys Leu Glu Val Trp
305                 310                 315
```

<210> 104
<211> 1265
<212> DNA
<213> Nannochloropsis oculata

<400> 104

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attttcagca aagtaatcaa gataataaac aaaaacaatc ctataaagga aaaacaacag        60

gcgcatcatc tacgacgata gccatggccg ccatctcacc gcgcaaacat cctccgcctg       120

atcttaagga gcgcatgatc gggggtctgc tcctggcttc gctcatctac gtatggctct       180

ttggtgtcat tgttgtaccc ttggctacgt acaagatgct ggcacagggc gactatcgcc       240

tcgccctcgg cctcctcctt tattacgcct accgttgggt ctatccgacc aaggaatggg       300

ccctcgtgcg cgacatctac cgagccggca accgatattt ctacccacaa gaggtccttt       360

ttgatggctt caaggagatc aaacccgaat cgaggtcatt gatttgcatg cacccgcatg       420

gaatcttgac tattggttgg gcgttgacca gcacgagtcc caccatgacg cacgccaatg       480

tgaagtggct ggtgacggag gctttgttgc gcttgccttt tatcagcgac ttcctgtcct       540

ggaacggctg tgcacacgct agcaagagct acatgcaaaa ccgtatgacg aagggtgcga       600

atcttgccct gctccccgga gggtttgaag aggcttccct ctatcaacac agctcttacc       660

gtgtctacat ccgaaagcgc acaggctttg ttgtgtatgc cctcagatat ggttataaga       720
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tttatccttc gttcgtcttt ggggaggaga agtgttattt ctctttgatg cccgactggg 780

ggtggctaac ggcggcgagg ctatggttga atcagttccg gttcccggca gttgcgtttg 840

tcggaaagtt gtttttggtg cctgggtggg attcgcattt gatcacggtg atcggcgccc 900

ccgtggtgtt gccgaggcta gagaagccaa cggaagagga ggtgaggaag taccattcgt 960

tgtatgtgcg tgcattgatg gaattgtttg agaagcacaa aacccaatat tgtgagaagg 1020

gggcgaagtt ggaggtgtgg taggataggg agagagggaa gggaaggtaa cacacatgta 1080

cagagctatg accaaagtaa tcgactgatg ggaggaggga gagggaaagt gaaagggaga 1140

aagaaagaga gaggggggagg ctgccacacc gcgacgctgc gtgagtgcgt ggtgtgtgtg 1200

tgtggagccc ttgatatttg aaataaaaat taaaaataaa aaaaaaaaaa aaaaaaaaaa 1260

aaaaa 1265

<210> 105
<211> 1563
<212> DNA
<213> Arabidopsis thaliana

<400> 105
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ctctctggtt ccgataataa ttctccttcg gatgatgttg gagctcccgc cgacgttagg 180

gatcggattg attccgttgt taacgatgac gctcagggaa cagccaattt ggccggagat 240

aataacggtg gtggcgataa taacggtggt ggaagaggcg gcggagaagg aagaggaaac 300

gccgatgcta cgtttacgta tcgaccgtcg gttccagctc atcggagggc gagagagagt 360

ccacttagct ccgacgcaat cttcaaacag agccatgccg gattattcaa cctctgtgta 420

gtagttctta ttgctgtaaa cagtagactc atcatcgaaa atcttatgaa gtatggttgg 480

ttgatcagaa cggatttctg gtttagttca agatcgctgc gagattggcc gcttttcatg 540

tgttgtatat ccctttcgat ctttcctttg gctgccttta cggttgagaa attggtactt 600

cagaaataca tatcagaacc tgttgtcatc tttcttcata ttattatcac catgacagag 660

gttttgtatc cagtttacgt caccctaagg tgtgattctg ctttttttatc aggtgtcact 720

ttgatgctcc tcacttgcat tgtgtggcta aagttggttt cttatgctca tactagctat 780

gacataagat ccctagccaa tgcagctgat aaggccaatc ctgaagtctc ctactacgtt 840

agcttgaaga gcttggcata tttcatggtc gctcccacat tgtgttatca gccaagttat 900

ccacgttctg catgtatacg gaagggttgg gtggctcgtc aatttgcaaa actggtcata 960

ttcaccggat tcatgggatt tataatagaa caatatataa atcctattgt caggaactca 1020

aagcatcctt tgaaaggcga tcttctatat gctattgaaa gagtgttgaa gctttcagtt 1080

ccaaatttat atgtgtggct ctgcatgttc tactgcttct tccacctttg gttaaacata 1140

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agtgtgggag attactggag aatgtggaat atgcctgttc ataaatggat ggttcgacat 1260

atatacttcc cgtgcttgcg cagcaagata ccaaagacac tcgccattat cattgctttc 1320

ctagtctctg cagtctttca tgagctatgc atcgcagttc cttgtcgtct cttcaagcta 1380

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gaaaggtttg gctcaacggt ggggaacatg atcttctggt tcatcttctg cattttcgga 1500

caaccgatgt gtgtgcttct ttattaccac gacctgatga accgaaaagg atcgatgtca 1560

tga 1563

<210> 106
<211> 520
<212> PRT
<213> Arabidopsis thaliana

<400> 106

Met Ala Ile Leu Asp Ser Ala Gly Val Thr Thr Val Thr Glu Asn Gly
1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg
20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser
35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp
50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp
65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu
85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro
100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe
115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile
130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp
145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp
165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala
180 185 190

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Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val
        195                 200                 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro
    210                 215                 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr
225                 230                 235                 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala
                245                 250                 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala
            260                 265                 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe
        275                 280                 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala
    290                 295                 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile
305                 310                 315                 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile
                325                 330                 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile
            340                 345                 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys
        355                 360                 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu
    370                 375                 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys
385                 390                 395                 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp
                405                 410                 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys
            420                 425                 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu
        435                 440                 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu
    450                 455                 460
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Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln
465                 470                 475                 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe
                485                 490                 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu
            500                 505                 510

Met Asn Arg Lys Gly Ser Met Ser
        515                 520
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<210> 107
<211> 1506
<212> DNA
<213> Brassica napus

<400> 107

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gtaactgttt ccgatgctga cgtgagggat cgggttgatt cagctgttga ggatactcaa       180

ggaaaagcca atttggccgg agaaaacgaa attagggaat ccggtggaga agcgggggga       240

aacgtggatg taaggtacac gtatcggccg tcggttccag ctcatcggag ggtgcgggag       300

agtccactca gctctgacgc catcttcaaa cagagccatg ctggactatt caacctgtgt       360

gtagtagttc ttgttgctgt aaacagtaga ctcatcatcg aaaatctcat gaagtacggt       420

tggttgatca gaactgattt ctggtttagt tcaacgtctc tgcgagattg gccccttttc       480

atgtgttgtc tctccctttc aatctttcct ttggctgcct ttaccgtcga gaaattagta       540

cttcagaaat gcatatctga acctgttgtc atcattcttc atattattat caccatgacc       600

gaggtcttgt atccagtcta tgtcactcta aggtgtgatt ccgccttctt atcaggtgtc       660

acgttgatgc tcctcacttg cattgtgtgg ctgaagttgg tttcttacgc tcatactaac       720

tatgacataa gaaccctagc taattcatct gataaggcca atcctgaagt ctcctactat       780

gttagcttga agagcttggc gtatttcatg cttgctccca cattgtgtta tcagccgagc       840

tatccacgtt ctccatgtat ccggaagggt tgggtggctc gtcaatttgc aaagctgatc       900

atattcactg gattcatggg atttataata gagcaatata taaatcctat tgttaggaac       960

tcaaaacatc ctttgaaagg ggatctctta tacggtgttg aaagagtgtt gaagctttca      1020

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atattggcag agctcctctg cttcggggat cgtgaattct acaaagattg gtggaatgca      1140

aaaagcgtgg gagattattg gagaatgtgg aatatgcctg ttcataaatg gatggttcga      1200

catgtatact ttccgtgcct tcgcagaaat ataccgaaag tacccgctat tatccttgct      1260

ttcttagtct ctgcagtctt tcatgagtta tgcatcgcag ttccttgtcg tctcttcaaa      1320

ctatgggctt tcttggggat tatgtttcag gtgcctttgg tatttatcac aaactaccta      1380
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caagaaaggt ttggctccat ggtgggaaac atgatattct ggtttacctt ctgcattttc 1440

ggacaaccga tgtgtgtgct tctttattat cacgacttga tgaaccgcaa aggaaagatg 1500

tcatag 1506

<210> 108
<211> 501
<212> PRT
<213> Brassica napus

<400> 108

Met Glu Ile Leu Asp Ser Gly Gly Val Thr Met Pro Thr Glu Asn Gly
1 5 10 15

Gly Ala Asp Leu Asp Thr Leu Arg His Arg Lys Pro Arg Ser Asp Ser
20 25 30

Ser Asn Gly Leu Leu Pro Asp Ser Val Thr Val Ser Asp Ala Asp Val
35 40 45

Arg Asp Arg Val Asp Ser Ala Val Glu Asp Thr Gln Gly Lys Ala Asn
50 55 60

Leu Ala Gly Glu Asn Glu Ile Arg Glu Ser Gly Gly Glu Ala Gly Gly
65 70 75 80

Asn Val Asp Val Arg Tyr Thr Tyr Arg Pro Ser Val Pro Ala His Arg
85 90 95

Arg Val Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe Lys Gln Ser
100 105 110

His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Val Ala Val Asn
115 120 125

Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp Leu Ile Arg
130 135 140

Thr Asp Phe Trp Phe Ser Ser Thr Ser Leu Arg Asp Trp Pro Leu Phe
145 150 155 160

Met Cys Cys Leu Ser Leu Ser Ile Phe Pro Leu Ala Ala Phe Thr Val
165 170 175

Glu Lys Leu Val Leu Gln Lys Cys Ile Ser Glu Pro Val Val Ile Ile
180 185 190

Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro Val Tyr Val
195 200 205

Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr Leu Met Leu

210 215 220

Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala His Thr Asn
225 230 235 240

Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ser Asp Lys Ala Asn Pro Glu
245 250 255

Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe Met Leu Ala
260 265 270

Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro Cys Ile Arg
275 280 285

Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Ile Ile Phe Thr Gly
290 295 300

Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile Val Arg Asn
305 310 315 320

Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Gly Val Glu Arg Val
325 330 335

Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys Met Phe Tyr
340 345 350

Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu Leu Cys Phe
355 360 365

Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys Ser Val Gly
370 375 380

Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met Val Arg
385 390 395 400

His Val Tyr Phe Pro Cys Leu Arg Arg Asn Ile Pro Lys Val Pro Ala
405 410 415

Ile Ile Leu Ala Phe Leu Val Ser Ala Val Phe His Glu Leu Cys Ile
420 425 430

Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu Gly Ile Met
435 440 445

Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln Glu Arg Phe
450 455 460

Gly Ser Met Val Gly Asn Met Ile Phe Trp Phe Thr Phe Cys Ile Phe
465 470 475 480

Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu Met Asn Arg

485 490 495

Lys Gly Lys Met Ser
500

<210> 109
<211> 104
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 109
ataaaagtat caacaaaaaa ttgttaatat acctctatac tttaacgtca aggagaaaaa 60

accccggatc ggcgcgccac catggccgcc atctcaccgc gcaa 104

<210> 110
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 110
aactataaaa aaataaatag ggacctagac ttcaggttgt ctaactcctt ccttttcggt 60

tagagcggat ttaattaact accacacctc caacttcgcc c 101

<210> 111
<211> 104
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 111
ataaaagtat caacaaaaaa ttgttaatat acctctatac tttaacgtca aggagaaaaa 60

accccggatc ggcgcgccac catggcgatt ttggattctg ctgg 104

<210> 112
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 112
aactataaaa aaataaatag ggacctagac ttcaggttgt ctaactcctt ccttttcggt 60

tagagcggat ttaattaatc atgacatcga tccttttcgg t 101

<210> 113
<211> 104
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 113
ataaaagtat caacaaaaaa ttgttaatat acctctatac tttaacgtca aggagaaaaa 60

accccggatc ggcgcgccac catggagatt ttggattctg gagg   104

<210> 114
<211> 101
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 114
aactataaaa aaataaatag ggacctagac ttcaggttgt ctaactcctt ccttttcggt 60

tagagcggat ttaattaact atgacatctt tcctttgcgg t   101