

using a QIAprep® Spin Miniprep kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocol.

The DNA molecules were then transformed into *Y. lipolytica* strain Y2224 as described in the General Methods, and the transformants were selected on MM plates. After 2 days growth at 30 °C, transformants selected on MM plates were picked and re-streaked onto fresh MM plates.

Quick Screen Plate Assay

A quick screen "plate assay" was used for the preliminary functional analysis of each mutant library. For this plate assay, transformant *Yarrowia* cells from the re-streaked MM plates above were analyzed directly from the media plate. Fatty acid methyl esters ["FAMES"] were prepared using trimethylsulphonium hydroxide ["TMSH"].

The TMSH was prepared from trimethylsulfonium iodide ["TMSI"], after conversion to a solution of the hydroxide by reaction with silver oxide in methanol. Specifically, 4.4 g TMSI were mixed in 100 mL MeOH and allowed to incubate in a 50 °C water bath for 1 hr; then, 5 g Ag₂O were added to the solution and stirred for 4 hr at room temperature. The final solution was filtered before use. TMSH causes base-catalyzed transesterification of O-acyl lipids (i.e., TAG) and esterification of free fatty acids (A. H. El-Hamdy & W. W. Christie, *J. of Chromatography*, 630:438-441 (1993)).

Using a 1 µl loop, cells were taken directly from the re-streaked MM plate and suspended in 50 µl TMSH in a gas chromatogram ["GC"] vial with a 0.35 mL insert. Heptane (150 µl) was then added to the vial insert, the vial was capped and then incubated for 20 min at room temperature with agitation. Subsequently, 1 µl from the heptane layer was injected into a Hewlett Packard 7890 GC fitted with an Omegawax 320 fused silica capillary column (Supelco Inc., Bellefonte, PA) for GC analysis of FAMES. Retention times were compared to those for methyl esters from commercial standards (Standard #461, Nu-Chek Prep, Inc., Elysian, MN).

The FAME profiles obtained from cells comprising the EgD9eS mutants were compared to that of the non-mutant EgD9eS control. The results of this primary screen served as the basis for the selection of mutants that would be subjected to the secondary confirmation assay. The criteria used to select mutants for the confirmation assay was based on the lipid profile, in particular the concentration of EDA as calculated from the GC peak area of the corresponding FAME as a percent relative to the sum of all integrated peaks ["EDA % TFAs"] and/or the conversion efficiency of LA to EDA. The conversion efficiency ["% Conv"] of LA to EDA was calculated for each transformant according to the following formula: $([\text{product}]/[\text{substrate} + \text{product}]) \times 100$, wherein the product was EDA % TFAs and the substrate was the concentration of LA as an area percent of TFAs ["LA % TFAs"].

"Confirmation" Assay

Mutants that demonstrated improvement in delta-9 elongation activity relative to the control via the quick screen "plate assay" were selected for subsequent confirmation assays.

Yarrowia transformed with mutants were first grown from fresh re-streaked MM plates and then each mutant was individually inoculated into triplicate cultures comprising 3 mL liquid MM at 30 °C and shaken at 250 rpm/min for 2 days. The cells were collected by centrifugation, lipids were extracted, and FAMES were prepared by transesterification of the lipid extract with sodium methoxide (Roughan, G., and Nishida I., *Arch. Biochem. Biophys.*, 276(1):38-46 (1990)) and subsequently analyzed by GC, as described for the plate assay (*supra*).

Following confirmation of improved delta-9 elongation activity, each mutant pZUFmEgD9ES plasmid was recovered from the transformed *Y. lipolytica* strain Y2224 from which it was expressed, using the Zymoprep™ Yeast Plasmid Miniprep II kit (Cat. No. D2004, Zymo Research, Orange, CA), as recommended by the manufacturer.

The sequence of the rescued plasmid was characterized using standard DNA sequencing methods. In brief, DNA sequence was generated

on an ABI Automatic sequencer using dye terminator technology (U.S. Pat. 5,366,860; EP 272,007) using a combination of vector and insert-specific primers. Comparisons of genetic sequences were accomplished using standard tools well known in the art.

EXAMPLE 3

Construction Of Two EgD9eS Error Prone PCR Libraries

The present Example describes the synthesis of two delta-9 elongase error prone polymerase chain reaction [“ePCR”] libraries. The two ePCR libraries were created in a two-step method that first required the generation of a suite of megaprimers comprising random mutations within the templates, followed by the use of these megaprimers to make point mutations into pZuFmEgD9ES. The construct pZuFmEgD9ES (SEQ ID NO:25) (Example 1) was used as the DNA template for the first ePCR library. The second ePCR library used hits from the first ePCR library as DNA templates.

Creation Of Megaprimers Using A Random Mutagenesis Kit

The GeneMorph II Random Mutagenesis Kit (Cat. No. 200550, Stratagene, La Jolla, CA) was used to create random amino acid substitutions in the target protein. It functions by introducing mutations into the target gene during error-prone PCR using a novel error prone PCR enzyme blended formation comprising a combination of two different polymerases to produce a less biased mutational spectrum with equivalent mutation rates at A's and T's versus G's and C's. It is advertized that mutation rates of 1–16 mutations per kB can be achieved using a single set of buffer conditions optimized for high product yield. The desired mutation rate can be controlled simply by varying the initial amount of template DNA in the reaction and/or the number of amplification cycles performed.

The above kit was utilized to generate EgD9eS “megaprimers”, using the protocol recommended by the manufacturer. These megaprimers were about 930 bp long and comprised the 777 bp encoding EgD9eS (SEQ ID NO:9). The reaction mixture contained either 16 ng of DNA template per μ l

for the first ePCR library or 2.0 ng of DNA template per μl for the second library. It also comprised reaction buffer, dNTPs (0.8 mM), primer pZUFm_6980_012208f (SEQ ID NO:26) (2 μM), primer pZUFm_210_012208r (SEQ ID NO:27) (2 μM) and Mutazyme® II DNA polymerase (0.25 U/ μl). The PCR reaction was performed in a thin well 200 μl tube in Mastercycler gradient equipment (Brinkmann Instruments, Inc., Westbury, NY). PCR amplification was performed using the following conditions: 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, and elongation at 72 °C for 90 sec. A final elongation cycle at 72 °C for 4 min was carried out, followed by reaction termination at 4 °C.

The PCR products were purified using a DNA Clean & Concentrator™-5 kit (Cat. No. D4003, Zymo Research, Orange, CA), as recommended by the manufacturer. The purified double-stranded PCR products were utilized as “megaprimers”, each containing various mutations within EgD9eS.

Standard Cloning Methods To Create ePCR Mutant Genes Of EgD9eS

For the first ePCR library, “megaprimers” were digested with *NcoI* and *NotI* restriction enzymes. The gel purified *NcoI/NotI* gene fragment was then directly ligated into gel purified *NcoI/NotI* pZUFmEgD9ES vector (SEQ ID NO:25) using T4 DNA ligase (Promega, Madison, WI), via a ligation reaction at room temperature for 5 hr.

Site-Directed Mutagenesis To Create ePCR Mutant Genes Of EgD9eS

To create the second ePCR library, the “megaprimers” described above were utilized in reactions designed to introduce the EgD9eS mutations within the “megaprimers” into pZuFmEgD9ES (FIG. 2; SEQ ID NO:25), thereby replacing the non-mutant EgD9eS gene with various mutant EgD9eS genes. This was accomplished using the QuikChange® II XL site directed mutagenesis kit (Cat. No. 200524, Stratagene, La Jolla, CA).

The QuikChange® II site-directed mutagenesis kit is used to make point mutations, replace amino acids, and delete or insert single/multiple adjacent amino acids within an insert of interest in a double-stranded vector,

using the high-fidelity *Pfu*Ultra DNA polymerase for mutagenic primer-directed replication of both plasmid strands. The kit requires no specialized vectors, unique restriction sites, or multiple transformations and allows site-specific mutation in virtually any double-stranded plasmid. The basic procedure utilizes two synthetic oligonucleotide primers, both containing the desired mutation and complementary to opposite strands of the vector, which are extended during temperature cycling by the high-fidelity DNA polymerase without primer displacement. Extension of the oligonucleotide primers generates a mutated plasmid containing staggered nicks, which is then treated with the *Dpn* I endonuclease. This restriction enzyme is specific for methylated and hemi-methylated DNA, thereby allowing digestion of the parental DNA template and selection for mutation-containing synthesized DNA. The nicked vector DNA containing the desired mutations is then transformed and propagated in an *Escherichia coli* host.

In the present methodology, however, the double-stranded megaprimers comprising various mutant EgD9eS genes were used in place of traditional synthetic oligonucleotide primers. Specifically, a 50 μ l reaction was prepared comprising 5.0 μ l of 10x kit-supplied reaction buffer, 1.0 μ l of 50 ng/ μ l pZUFmEgD9ES template (SEQ ID NO:25), 42 μ l megaprimer, 1.0 μ l of 40 mM kit-supplied dNTP mix and 1.0 μ l kit-supplied *Pfu*-Ultra DNA polymerase. This reaction mixture was placed in a thin well 200 μ l-capacity PCR tube and subjected to PCR amplification, using the following conditions: 95 °C for 30 sec, followed by 25 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 1 min, and elongation at 68 °C for 6 min. A final elongation cycle at 68 °C for 8 min was carried out, followed by reaction termination at 4 °C.

Kit-supplied *Dpn*I restriction enzyme (1.0 μ l) was directly added to the finished site-directed mutagenesis reaction mixture and enzymatic digestion was performed at 37 °C for 1 hr to remove the DNA template. The digested product was purified using a DNA cleaning kit (Zymo Research) and eluted to

yield 10 µl of purified DNA, comprising various mutant EgD9eS genes contained within the pZUFmEgD9ES vector backbone.

EXAMPLE 4

Identification of ePCR EgD9eS Library Mutants Having Improved Delta-9 Elongase Conversion Efficiency

The present Example describes: 1) the identification of EgD9eS ePCR library mutants having improved delta-9 elongase conversion efficiency of LA to EDA, as compared to that of the wildtype protein EgD9eS (SEQ ID NO:10); and, 2) sequence analysis of these EgD9eS ePCR library mutants.

Identification Of EgD9eS ePCR Mutants

The ePCR gene library mutants prepared in Example 3 were transformed into *E. coli* Top 10 electro-competent cells, purified and subsequently transformed into *Y. lipolytica* strain Y2224, as described in Example 2. The fatty acid profiles of 1,724 *Yarrowia* transformants were screened using the quick screen “plate assay” of Example 2. Most of these mutants exhibited reduced activity compared to the control. However, five transformants were confirmed to exhibit improved delta-9 elongation activity as compared to the control, based on confirmation assays of Example 2.

Data from two independent confirmation assays are presented in Table 5 and Table 6, and the FAME profiles of individual pZuFmEgD9ES control transformants are compared with those of ePCR mutants. More specifically, the concentration of each fatty acid as calculated from the GC peak area of the corresponding FAME as a percent relative to the sum of all integrated peaks [% TFAs] and % Conv. of LA to EDA (determined as described in Example 2) for each strain is shown below in Table 5 and Table 6, while averages are highlighted in gray and indicated with “Avg”. Fatty acids are identified as 16:0 (palmitic acid), 16:1 (palmitoleic acid), 18:0 (stearic acid), 18:1 (oleic acid), LA and EDA. Comparison of each mutant’s performance relative to the EgD9eS control should only be made within the particular confirmation assay in which each mutant was analyzed (i.e., comparisons can not be made between Assay #1 and Assay #2).

Table 5. Confirmation Assay #1: Lipid Composition In Transformant Y.
lipolytica Strain Y2224, Expressing EgD9eS Or ePCR Library Mutant Variants
Thereof

Strain	Replicate No.	% TFAs						% Conv LA to EDA
		16:0	16:1	18:0	18:1	LA	EDA	
EgD9eS Control-1	1	12.8	12.9	3.0	46.6	17.3	3.0	14.8
	2	12.9	12.6	3.0	45.5	17.2	3.1	15.2
	3	12.6	12.5	2.9	47.0	17.1	3.1	15.3
EgD9eS Control-2	1	14.0	12.4	3.6	45.6	16.2	3.2	16.3
	2	12.2	12.5	2.6	47.4	17.3	3.1	15.1
	3	13.2	13.1	3.1	45.4	17.2	3.1	15.3
EgD9eS Control-3	1	13.3	13.2	3.1	45.0	17.2	3.2	15.7
	2	12.8	12.6	2.9	46.5	17.3	3.1	15.0
	3	13.2	13.0	3.1	45.3	17.2	3.2	15.7
EgD9eS Control-4	1	13.7	11.9	3.7	46.2	15.8	3.4	17.6
	2	12.6	13.0	2.7	45.5	17.9	3.2	15.3
	3	12.7	12.9	2.9	45.6	17.6	3.2	15.5
EgD9eS Control-5	1	12.9	12.6	3.0	45.7	17.7	3.1	14.9
	2	12.1	12.1	2.7	47.9	17.3	3.1	15.2
Avg. Control	--	12.9	12.7	3.0	46.1	17.2	3.1	15.5
1.2ep-8	1	11.8	12.6	2.4	47.7	17.1	3.6	17.3
	2	12.1	12.9	2.5	47.0	16.9	3.7	17.9
	3	12.7	12.8	2.9	45.9	16.9	3.7	18.0
	Avg	12.2	12.8	2.6	46.9	17.0	3.7	17.8
1.9ep-63	1	12.5	12.9	2.7	46.1	17.5	3.3	15.9
	2	12.6	12.7	2.8	46.2	17.3	3.3	16.0
	3	13.0	12.6	3.2	45.7	16.9	3.4	16.8
	Avg	12.7	12.7	2.9	46.0	17.2	3.3	16.3
1.4ep-161	1	13.7	12.3	3.5	45.8	16.2	3.3	17.0
	2	12.4	12.7	2.9	46.9	16.8	3.2	16.0
	3	12.5	12.4	3.0	46.8	16.9	3.3	16.3
	Avg	12.9	12.5	3.1	46.5	16.6	3.3	16.4

Table 6. Confirmation Assay #2: Lipid Composition In Transformant Y.
lipolytica Strain Y2224, Expressing EgD9eS Or ePCR Library Mutant Variants
Thereof

Strain	Replicate No.	% TFAs						% Conv LA to EDA
		16:0	16:1	18:0	18:1	LA	EDA	
EgD9eS	1	12.0	12.1	3.0	50.2	14.0	2.9	16.9

Control-2	2	12.0	11.6	3.1	50.4	14.0	2.7	16.0
	3	11.8	12.1	3.0	51.1	14.4	2.8	16.3
EgD9eS Control-5	1	11.8	12.1	3.2	50.6	13.7	3.2	18.9
	2	11.9	12.2	3.2	51.0	13.9	3.3	19.2
	3	11.8	11.8	3.2	51.8	13.9	2.9	17.1
EgD9eS Control-6	1	11.8	11.7	3.2	51.1	14.0	2.8	16.6
	2	11.8	11.9	3.3	51.0	14.5	2.8	16.4
	3	11.6	12.2	2.8	51.3	14.9	2.8	15.8
EgD9eS Control-7	1	11.9	11.8	3.4	51.1	14.3	2.8	16.2
	2	11.8	12.0	3.2	51.1	14.2	2.8	16.6
	3	12.0	12.0	3.2	50.8	14.1	2.8	16.5
Avg. Control	--	11.9	11.9	3.2	51.0	14.1	2.9	16.9
2.1ep-94	1	11.7	11.1	2.8	50.0	14.9	3.5	19.2
	2	10.8	11.8	2.0	50.5	15.8	3.9	19.9
	3	11.1	11.5	2.0	51.2	15.3	3.9	20.2
	Avg	11.2	11.5	2.3	50.5	15.3	3.8	19.8
2.1ep-95	1	11.8	10.9	2.7	50.4	15.1	3.5	18.9
	2	11.8	11.0	2.6	50.6	15.5	3.5	18.6
	3	12.0	11.0	3.2	50.1	15.1	3.5	18.7
	Avg	11.9	11.0	2.8	50.4	15.2	3.5	18.8

In summarizing the data shown above in confirmation assay #1, clones of *Y. lipolytica* strain Y2224 that were transformed with pZuFmEgD9ES, comprising the non-mutant codon-optimized EgD9eS gene, produced an average of 3.1 EDA % TFAs, wherein the average conversion efficiency ["% Conv"] of LA to EDA in these five clones was determined to be about 15.5%. In contrast, the average % Conv of LA to EDA for mutant strain 1.2ep-8 was 17.8% (or 115% relative to the control); the average % Conv for mutant strain 1.9ep-63 was 16.3% (or 105% relative to the control); and, the average % Conv for mutant strain 1.4ep-161 was 16.4% (or 106% relative to the control).

In confirmation assay #2, clones of *Y. lipolytica* strain Y2224 that were transformed with pZuFmEgD9ES produced 2.9 EDA % TFAs, wherein the average % Conv of LA to EDA in these four strains was determined to be about 16.9%. The average % Conv of LA to EDA for mutant strain 2.1ep-94 was 19.8% (or 117% relative to the control); and, the average % Conv for mutant strain 2.1ep-95 was 18.8% (or 111% relative to the control).

Thus, these experiments confirmed the improved delta-9 elongase conversion efficiency exhibited by EgD9eS ePCR mutants 1.2ep-8, 1.9ep-63, 1.4ep-161, 2.1ep-94 and 2.1ep-95.

Sequence Of EgD9eS ePCR Mutants

The plasmids rescued from mutants 1.2ep-8, 1.9ep-63, 1.4ep-161, 2.1ep-94 and 2.1ep-95 were characterized by DNA sequencing, and analysis revealed various nucleotide substitutions and expressed amino acid substitutions within the mutant EgD9eS genes, as shown in Table 7. A designation indicative of the amino acid substitution was given to each mutant EgD9eS gene and to each mutant pZuFmEgD9ES plasmid comprising the mutant EgD9eS gene. For each substitution listed (i.e. L35G), the first letter corresponds to the amino acid in the non-mutant EgD9eS (i.e., SEQ ID NO:10) and the second letter corresponds to the amino acid found in the same position in the mutant, i.e. L35G indicates a change from Leu in EgD9eS at position 35 to Gly in the EgD9eS mutant).

Table 7. Summary of Sequenced EgD9eS ePCR Library Mutants

ePCR Mutant	Nucleotide Substitution	Resulting Amino Acid Substitution (Silent Mutation)	Designation For Mutant Gene	Designation For Mutant Plasmid
1.2ep-8	C103T and A654G	L35F and (G218G)	"EgD9eS-L35F" (SEQ ID NO:28)	pZuFmEgD9ES-L35F (SEQ ID NO:30)
1.9ep-63	A173G, T234G, G402A and T770C	K58R, (S78S), (Q134Q) and I257T	"EgD9eS-K58R/I257T" (SEQ ID NO:31)	pZuFmEgD9eS-K58R/I257T (SEQ ID NO:33)
1.4ep-161	C388A, C450T and T728C	L130M, (N150N) and V243A	"EgD9eS-L130M/V243A ₁ " (SEQ ID NO:34)	pZuFmEgD9ES-L130M/V243A ₁ (SEQ ID NO:36)
2.1ep-95	A293G	D98G	"EgD9eS-D98G" (SEQ ID NO:37)	pZuFmEgD9ES-D98G (SEQ ID NO:39)
2.1ep-94	C21T, C388A, C450T	(I7I), L130M, (N150N)	"EgD9eS-L130M/V243A ₂ " (SEQ ID NO:40)	pZuFmEgD9ES-L130M/V243A ₂ (SEQ ID NO:42)

	and T728C	and V243A		
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Thus, for example, the plasmid rescued from mutant 1.2ep-8 comprised 2 nucleotide substitutions (i.e., C103T and A654G). These two nucleotide substitutions correspond to one expressed amino acid substitution (i.e., L35F), and one silent amino acid mutation (i.e., G218G; since both GGA and GGG code for Gly, this amino acid was unchanged in the mutant protein as a result of the A654G nucleotide substitution). The plasmid comprising the C103T and A654G mutations, resulting in the amino acid change L35F, was designated as pZuFmEgD9ES-L35F (SEQ ID NO:30), while the nucleotide sequence of the mutant delta-9 elongase therein is designated as "EgD9eS-L35F" (SEQ ID NO:28), having a protein sequence as set forth in SEQ ID NO:29.

EXAMPLE 5

Construction of A Two-Site-Saturation EgD9eS Gene Library

The present example describes the synthesis of a site-saturation ["SS"] library, prepared by targeting amino acid positions 35 and 107 within EgD9eS (SEQ ID NO:10). The rationale for targeting position 35 was based on the results of Example 4, while the rationale for targeting position 107 is described below. The SS library was created in a two-step method that first required the generation of megaprimers comprising targeted mutations within the template, followed by use of these megaprimers to make point mutations into pZuFmEgD9ES.

Rationale For Targeting Position 107 Of EgD9eS

First, the amino acid sequences of 17 fatty acid elongases, as described in Table 8 below, were aligned using the ClustalW method of alignment.

Table 8. Fatty Acid Elongases Subjected To Conservation Pattern Analysis

Elongase Abbreviation	Organism	Reference	SEQ ID NO
Ci_elo	<i>Ciona intestinalis</i>	GenBank Accession No. AAV67802	43
Om_elo	<i>Oncorhynchus mykiss</i>	GenBank Accession No. AAV67803	44
Mp_elo1	<i>Marchantia polymorpha</i>	GenBank Accession No. AAT85662	45
Pp_elo1	<i>Physcomitrella patens</i>	GenBank Accession No. AAL84174	46
Mp_d5e	<i>Marchantia polymorpha</i>	GenBank Accession No. BAE71130	47
Ot_elo1	<i>Ostreococcus tauri</i>	GenBank Accession No. AAV67797	48
Pav_elo2	<i>Pavlova</i> sp. CCMP459	GenBank Accession No. AAV33630	49
Ps_elo2	<i>Pavlova salina</i>	GenBank Accession No. AAY15135	50
Ot_elo2	<i>Ostreococcus tauri</i>	GenBank Accession No. AAV67798	51
Ea_d9e	<i>Euglena anabaena</i>	U.S. Pat. 7,794,701	12
Eg_d9e	<i>Euglena gracilis</i>	U.S. Pat. 7,645,604	8
E398_d9e	<i>Eutreptiella</i> sp. CCMP389	U.S. Pat. 7,645,604	4
Ig_d9e	<i>Isochrysis galbana</i>	PCT Publications No. WO 2002/077213, No. WO 2005/083093, No. WO 2005/012316 and No. WO 2004/057001; GenBank Accession No. AAL37626	2
Tp_elo2	<i>Thalassiosira pseudonana</i>	GenBank Accession No. AAV67800	52
Tp_elo1	<i>Thalassiosira pseudonana</i>	GenBank Accession No. AAV67799	53
Ma_d6e	<i>Mortierella alpina</i>	GenBank Accession No. AAF70417	54
Th_elo2	<i>Thraustochytrium</i> sp. FJN-10	GenBank Accession No. ABC18314	55

The Clustal W alignment method, described by Thompson et al. (*Nucleic Acids Res.* 22:4673-4680 (1994)), was performed using a ClustalW package (Version 1.83) with default parameters (i.e., protein weight matrix = Gonnet 250, gap opening penalty = 10, gap extension penalty=0.2 and full alignment algorithm). Results of the alignment are shown in FIG. 3 (comprising FIGs. 3A, 3B, 3C, 3D, 3E, 3F, 3G and 3H). "Trace_1", "Trace_2", "Trace_3" and "Trace_4" represent the consensus of each column for functional Group I, Group II, Group III and Group IV, as defined *infra*, i.e.,

Trace 1 represents the consensus of the protein sequences in Group I, comprising Ci_elo, Om_elo, Mp_elo1, Pp_elo1, Mp_d5e and Ot_elo1. The consensus of each column was defined as follows. Specifically, if the column was completely conserved, then the consensus was represented as the conserved amino acid, shown as a capital letter. If the column was conserved in terms of physio-chemical properties, then the consensus was represented with a lower case letter, wherein “k” represents amino acids D and E (negatively-charged), “q” represents amino acids H, K and R (positively-charged), “p” represents amino acids N and Q (polar), “a” represents amino acids I, L and V (aliphatic), “d” represents amino acids F, W and Y (aromatic), “h” represents amino acids A and G (tiny), “s” represents amino acids D, E, N, Q, H, K, R, S and T (hydrophilic) and “f” represents amino acids I, L, V, F, W, Y, C and M (hydrophobic). If the column was not conserved, then the consensus was represented with a capital letter “X”.

A neighbor-joining tree was generated from the Clustal W alignment. Based on the tree topology, the 17 sequences were partitioned into 4 groups, which are hypothesized to correspond to functional groups of different substrate specificity: Group I comprises Ci_elo, Om_elo, Mp_elo1, Pp_elo1, Mp_d5e and Ot_elo1; Group II comprises Pav_elo2, Ps_elo2 and Ot_elo2; Group III comprises Ea_d9e, Eg_d9e, E398_d9e and Ig_d9e; and, Group IV comprises Tp_elo2, Tp_elo1, Ma_d6e and Th_elo2.

Considering the alignment of FIG. 3 and the groupings of the neighbor-joining tree, the following conclusions were drawn. First, some positions are absolutely conserved across all 17 sequences within Group I, II, III and IV. These positions were considered to likely be essential for the catalytic activity of the elongase, and thus were eliminated as targets for mutation. Some positions were conserved in only some of the sequences within Group I, II, III and IV (i.e., not absolutely conserved). These positions were considered to likely be important for the substrate specificity exhibited by elongases within the functional groups of Group I, II, III or IV. Some positions were relatively conserved within Group III (comprising all four of the known delta-9

elongases), but variations were also exhibited; see, amino acid positions 22, 47, 54, 101, 107, 111, 115, 161, 182, 192 and 242, based on numbering of EgD9e. These positions were considered to likely be important for the activity of delta-9 elongases, and were hypothesized to modulate the differences in substrate specificity of Ea_d9e (SEQ ID NO:12), Eg_d9e (SEQ ID NO:8), E398_d9e (SEQ ID NO:4) and Ig_d9e (SEQ ID NO:2).

An analysis of the transmembrane ["TM"] domains within EgD9eS was performed using the TMHMM program ("Prediction of transmembrane helices in proteins"; TMHMM Server v. 2.0, Center for Biological Sequence Analysis, BioCentrum-DTU, Technical University of Denmark, DK-2800 Lyngby, Denmark). The prediction indicated six membrane-spanning helices (corresponding to amino acid residues 32-51, 66-88, 114-136, 156-175, 188-206, 221-243), with both N-and C- termini located on the cytoplasmic side. When Ot_elo2, Ig_elo1, Pav_elo2 and Tp_elo2 were similarly analyzed using the TMHMM program, the number of membrane-spanning helices varied from 4 to 8. Thus, in order to consolidate these varying predictions, the following pieces of functional information were used.

1. The highly conserved histidine-rich motif [Q/H]xxHH ("His-box") has been shown to be essential for optimum enzyme activity of Ig_d9e (SEQ ID NO:2), but is not directly responsible for substrate specificity (Qi et al., *FEBS Letters*, 547:137-139 (2003)). Thus, it strongly suggests that the His-box (corresponding to amino acid residues 134-138 in EgD9eS) is involved in the active site; and, it should be located in or near the cytosolic side of the folded protein such that substrate can access the active site.

2. Several highly conserved positions with charged residues are present at the C-terminal end of EgD9eS. They are likely relevant for the activity and thus the C-terminus is probably located in the cytosolic side of the folded protein.

In contrast to the TMHMM results which predicted a membrane-spanning helix between amino acid residues 114-136 and between amino acid residues 156-175, the above considerations indicate that the sequence region between residues 114-136 does not span the membrane since the His-box cannot be located in the external face of the membrane. If the C-terminus is located in the cytosolic side, then the predicted TM domain between 156-175 also does not span the membrane. Because the substrate for the elongase is highly hydrophobic, it will likely partition into the lipid bilayer. The active site (including the His-box) may occur at or very near the membrane surface.

Therefore, it is predicted herein that these two hydrophobic regions (i.e., corresponding to amino acid residues 114-136 and amino acid residues 156-175) lie in or near the inner membrane leaflet to ensure the active site sits close to the membrane. The final membrane topology model predicted for EgD9eS is shown in FIG. 4A. Specifically, each vertical cylinder indicates a membrane-spanning segment, while each horizontal cylinder indicates a hydrophobic stretch that lies in or near the inner membrane leaflet. The conserved glutamine [Q] and histidines [H] within the His-box (i.e., corresponding to amino acid residues 134-138) are indicated with small circles. Finally, "in" corresponds with the cytoplasmic space, while "out" corresponds with the periplasmic space.

While conservation pattern analysis identified 11 different amino acid residues within the Group III delta-9 fatty acid elongases (i.e., Ea_d9e [SEQ ID NO:12], Eg_d9e [SEQ ID NO:8], E398_d9e [SEQ ID NO:4] and Ig_d9e [SEQ ID NO:2]) that were predicted to affect enzyme activity, the results from the predicted topology model further limited candidate residues. Specifically, it was reasoned that positions that were important for enzymatic activity had to be on or near the cytosolic side, where the active site lies. Amino acid residues 47, 54 and 192 failed to meet this criterion and thus it was assumed that they could not be important for modulating the activity of the delta-9 elongases.

Based on the above rationale, the candidate residues that were likely to significantly impact delta-9 elongase activity of EgD9eS were reduced from 258 residues within the full-length protein of SEQ ID NO:10 to only 8 residues, corresponding to positions 22, 101, 107, 111, 115, 161, 182 and 242. These eight positions were recommended as targets for site-directed mutagenesis to improve the substrate conversion rate of EgD9eS. The experimental data below targeted position 107.

Creation Of Megaprimers For Construction Of The Site-Saturation Library

Oligonucleotides EgD9E_102_053008f (SEQ ID NO:56) and EgD9E_760_053008r (SEQ ID NO:57) were designed to target amino acid residues 35 and 107, respectively, of EgD9eS (SEQ ID NO:10). Following commercial synthesis of these oligonucleotides, they were utilized in a PCR reaction to create suitable megaprimers for use in the construction of the SS library. Specifically, a 50 µl reaction mixture was prepared to contain: 5.0 µl of 10x reaction buffer supplied with Pfu-*Ultra* polymerase (Stratagene), 1.0 µl of 50 ng/µl EgD9eS (SEQ ID NO:10), 1.0 µl of 10 pmol/µl primer EgD9E_102_053008f (SEQ ID NO:56), 1.0 µl of 10 pmol/µl primer EgD9E_760_053008r (SEQ ID NO:57), 1.0 µl of 40 mM dNTP mix (Promega, Madison, WI), 1.0 µl high fidelity Pfu-*Ultra* DNA polymerase (Stratagene) and 40 µl water. The mixture was placed in a thin well 200 µl tube for the PCR reaction in Mastercycler gradient equipment (Brinkmann Instruments, Inc. Westbury, NY). PCR amplification was performed using the following conditions: 95 °C for 30 sec, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 54 °C for 1 min, and elongation at 72 °C for 2 min. A final elongation cycle at 72 °C for 4 min was carried out, followed by reaction termination at 4 °C.

The PCR products were purified using a DNA Clean & Concentrator™-5 kit (Cat. No. D4003, Zymo Research, Orange, CA), as recommended by the manufacturer. The purified double-stranded PCR products were utilized as “megaprimers”, each containing various mutations within EgD9eS.

Site-Directed Mutagenesis To Create Site-Saturation Mutant Genes Of EgD9eS

The “megaprimers” described above were then utilized in reactions designed to introduce the EgD9eS mutations within the “megaprimers” into pZuFmEgD9ES (FIG. 2; SEQ ID NO:25), thereby replacing the non-mutant EgD9eS gene with various mutant EgD9eS genes. This was accomplished using the QuikChange® II XL site directed mutagenesis kit (Cat. No. 200524, Stratagene, La Jolla, CA), as described in Example 3. Specifically, the composition of the site directed mutagenesis reaction and amplification conditions were identical to that described in Example 3, as was the method of *DpnI* restriction and DNA clean-up.

EXAMPLE 6

Identification of EgD9eS Site-Saturation Library Mutants Having Improved Delta-9 Elongase Conversion Efficiency

The present Example describes: 1) the identification of EgD9eS mutants having improved delta-9 elongase conversion efficiency of LA to EDA, as compared to that of the wildtype protein EgD9eS (SEQ ID NO:10); and, 2) sequence analysis of these EgD9eS mutants.

Identification Of EgD9eS Site-Saturation Mutants

The SS library prepared in Example 5 was transformed into *E. coli* Top 10 electro-competent cells, purified and subsequently transformed into *Y. lipolytica* strain Y2224, as described in Example 2. The fatty acid profiles of 510 *Yarrowia* transformants with constructs from the SS library were analyzed using the quick screen “plate assay” of Example 2. Three transformants were confirmed to exhibit improved delta-9 elongation activity as compared to the control, based on the confirmation assay of Example 2.

Data from the confirmation assay is presented in Table 9, and the FAME profiles of individual pZuFmEgD9ES control transformants are compared with those of SS library mutants. More specifically, the concentration of each fatty acid as an area percent of TFAs [% TFAs] and %

Conv. of LA to EDA (determined as described in Example 2) for each strain is shown below in Table 9, while averages are highlighted in gray and indicated with "Avg". Fatty acids are identified based on the abbreviations of Example 4.

Table 9. Confirmation Assay: Lipid Composition In Transformant *Y. lipolytica* Strain Y2224, Expressing EgD9eS Or SS Library Mutant Variants Thereof

Strain	Replicate No.	% TFAs						% Conv LA to EDA
		16:0	16:1	18:0	18:1	LA	EDA	
EgD9eS Control-1	1	12.6	11.5	5.2	47.0	15.0	3.4	18.3
	2	12.5	11.8	4.9	47.1	15.1	3.4	18.4
EgD9eS Control-2	1	12.7	11.5	4.2	48.5	15.4	3.4	18.0
	2	12.0	12.0	4.1	47.1	16.9	3.8	18.2
	3	12.9	11.3	4.4	48.3	15.4	3.4	18.0
EgD9eS Control-3	1	12.5	11.7	3.7	49.4	15.5	3.5	18.6
	2	12.4	11.6	5.1	47.8	15.0	3.4	18.6
	3	12.1	11.8	5.0	48.3	15.4	3.5	18.6
EgD9eS Control-4	1	12.3	11.5	5.2	47.7	15.0	3.7	19.8
	2	12.4	11.8	4.7	48.1	15.0	3.5	19.1
	3	12.7	11.7	3.8	48.7	15.1	3.7	19.8
Avg. Control	--	12.5	11.6	4.6	48.0	15.3	3.5	18.7
2.4sd-24	1	12.6	11.8	4.0	48.6	13.3	4.9	27.0
	2	12.6	11.6	4.6	48.6	13.0	4.9	27.5
	3	12.5	11.8	3.9	49.6	13.2	4.9	27.0
	Avg	12.6	11.7	4.1	48.9	13.2	4.9	27.2
2.4sd-52	1	12.6	11.5	3.8	50.3	13.2	4.7	26.4
	2	12.5	11.2	4.3	49.4	13.2	4.7	26.2
	3	12.6	11.2	5.0	48.7	12.8	4.8	27.2
	Avg	12.6	11.3	4.4	49.4	13.1	4.7	26.6
2.4sd-53	1	12.6	12.0	3.6	50.1	13.8	4.5	24.8
	2	12.3	12.0	3.8	49.1	14.4	4.6	24.3
	3	12.5	12.4	3.6	49.2	13.6	4.4	24.6
	Avg	12.5	12.1	3.7	49.5	13.9	4.5	24.6

In the confirmation assay, clones of *Y. lipolytica* strain Y2224 that were transformed with pZuFmEgD9ES, comprising the non-mutant codon-optimized EgD9eS gene, produced an average of 3.5 EDA % TFAs, wherein the average conversion efficiency ["% Conv"] of LA to EDA in these four strains was determined to be about 18.7%. By comparison, the average %

Conv of LA to EDA for mutant strain 2.4sd2-24 was 27.2% (or 145% relative to the control); the average % Conv for mutant strain 2.4sd2-52 was 26.6% (or 142% relative to the control); and, the average % Conv for mutant strain 2.4sd2-53 was 24.6% (or 132% relative to the control). This assay therefore confirmed the improved delta-9 elongase conversion efficiency exhibited by site-saturation mutants 2.4sd2-24, 2.4sd2-52 and 2.4sd2-53.

Sequence Of EgD9eS Site-Saturation Mutants

The plasmids rescued from mutants 2.4sd-24, 2.4sd-52 and 2.4sd-53 were characterized by DNA sequencing, and analysis revealed various nucleotide substitutions and expressed amino acid substitutions within the mutant EgD9eS genes, as shown in Table 10. A designation indicative of the amino acid substitution was given to each mutant EgD9eS gene and to each mutant pZuFmEgD9ES plasmid comprising the mutant EgD9eS gene.

Table 10. Summary of Sequenced EgD9eS SS Library Mutants

Site-Saturation Mutant	Nucleotide Substitution	Resulting Amino Acid Substitution	Designation For Mutant Gene	Designation For Mutant Plasmid
2.4sd-24	C103G, T104G, C105G	L35G	"EgD9eS-L35G" (SEQ ID NO:58)	pZuFmEgD9ES-L35G (SEQ ID NO:60)
2.4sd-52	C103G, T104G, C105G	L35G		
2.4sd-53	C103A, C105G, C319G	L35M and Q107E	"EgD9eS-L35M/Q107E" (SEQ ID NO:61)	pZuFmEgD9ES-L35M/Q107E (SEQ ID NO:63)

As will be obvious to one of skill in the art, the Applicants appreciate that a variety of nucleotide sequences can encode, e.g., the protein set forth as EgD9eS-L35G, based on the degeneracy of the genetic code. Thus, for example, the Gly encoded in the mutant protein set forth as SEQ ID NO:59 at amino acid residue position 35 can be encoded by GGG (as in the delta-9 elongase open reading frame ["ORF"] set forth in SEQ ID NO:58), GGA

(as in the delta-9 elongase ORF set forth in SEQ ID NO:95), GGC (as in the delta-9 elongase ORF set forth in SEQ ID NO:96) and GGT (as in the delta-9 elongase ORF set forth in SEQ ID NO:97). A variety of other nucleotide substitutions that result in silent mutations in the encoded protein are also contemplated, and thus the nucleotide sequences provided herein which encode EgD9eS-L35G (SEQ ID NO:59) should not be construed as a limitation to the present disclosure. Similar variation is contemplated within any of the nucleotide sequences described herein, encoding the mutant proteins of the invention and having delta-9 elongase activity.

EXAMPLE 7

Creation Of EgD9eS-L35G SlonoMax® Libraries

The present example describes the synthesis of SlonoMax® libraries, prepared by targeting 50 distinct amino acid positions within the EgD9eS-L35G mutant (SEQ ID NO:59; Example 6), which demonstrated a 42-45% improvement in LA to EDA conversion efficiency when compared to the parent enzyme. Thus, this Example sought to identify additional beneficial mutations that could be “stacked” into the EgD9eS mutant comprising the L35 mutation.

Slonomics®, an automated robotic platform described in additional detail *infra*, generates SlonoMax® libraries where the number of mutants per sequence position and their ratios can be very precisely controlled. Thus, the automated process offers advantages in that the number of candidate residues that could be experimentally examined to determine their impact on delta-9 elongase activity could be greatly increased, as opposed to the limited residues considered upon creation of the site-saturation library (Example 5).

Rationale For Targeting 50 Distinct Residues Within EgD9eS For Functional Site Evaluation

Delta-9 elongase enzymes have been identified and functionally characterized from *Isochrysis galbana* [“IgD9e”] (SEQ ID NO:2; PCT

Publications No. WO 2002/077213, No. WO 2005/083093, No. WO 2005/012316 and No. WO 2004/057001; GenBank Accession No. AAL37626), *Eutreptiella* sp. CCMP389 ["E389D9e"] (SEQ ID NO:4; U.S. Pat. 7,645,604), *Euglena gracilis* ["EgD9e"] (SEQ ID NO:8; U.S. Pat. 7,645,604) and *E. anabaena* ["EaD9e"] (SEQ ID NO:12; U.S. Pat. 7,794,701). Each of these elongases has been shown to be capable of converting LA to EDA. EgD9e, EaD9e and E389D9e share more than 60% sequence similarity with one another, while IgD9E shares only about 35% sequence similarity to any one of EgD9e, EaD9e, and E389D9e (based on ClustalW (Version 1.83) analyses, using default parameters (i.e., protein weight matrix = Gonnet 250, gap opening penalty = 10, gap extension penalty=0.2 and full alignment algorithm).

It was observed that positions leading to mutants with improved delta-9 elongase conversion efficiency (e.g., D98G [Example 4] and L35G [Example 6]) have moderate sequence conservativeness. An amino acid sequence alignment of IgD9e, EgD9e, EaD9e and E389D9e was created to identify other moderately conserved residues, using default parameters of Vector NTI[®]'s AlignX program (Invitrogen Corporation, Carlsbad, CA) (FIG. 1). It was hypothesized that these moderately conserved residues might be good candidates as targets for amino acid substitution to potentially yield a second generation of mutant enzymes having improved activity relative to the non-mutant EgD9eS control.

Comparing the sequence of these four homologous enzymes, 58 of the 258 amino acid positions were determined to be conserved among all four elongase enzymes; thus, these residues were eliminated from consideration. Additionally, 92 positions were determined to be conserved between EgD9e, EaD9e and E389D9e; these positions were also eliminated from consideration. Lastly, since positions having random amino acid changes among homologs normally do not play a significant role in protein function, an additional 22 positions determined to possess four different amino acid

residues among all four elongase enzymes were thus eliminated from consideration as targeted positions for functional evaluation.

The remaining 86 positions within SEQ ID NO:8 (i.e., positions 1, 3, 4, 5, 9, 12, 21, 22, 27, 28, 29, 32, 35, 37, 41, 42, 45, 47, 48, 51, 52, 53, 54, 57, 58, 60, 62, 63, 66, 67, 70, 71, 73, 74, 80, 83, 84, 85, 89, 94, 98, 101, 104, 105, 107, 108, 111, 115, 127, 131, 132, 143, 149, 152, 153, 155, 156, 161, 168, 169, 179, 181, 182, 192, 196, 204, 207, 209, 210, 211, 216, 218, 222, 223, 225, 229, 236, 239, 242, 244, 245, 247, 250, 254, 257 and 258) were considered as potential targets for functional site evaluation. A comparison of the amino acid residue that is encoded at each one of these positions in EgD9e (SEQ ID NO:8), EaD9e (SEQ ID NO:12) and E389E9e (SEQ ID NO:4) is shown below in Table 11.

Table 11. Positions For Functional Site Evaluation

Position*	EgD9e	EaD9e	389D9e	Position*	EgD9e	EaD9e	389D9e
1	M	M	I	105	T	T	A
3	V	A	V	107	Q	Q	K
4	V	A	A	108	L	L	V
5	N	K	N	111	L	L	Y
9	S	S	A	115	L	V	L
12	Q	Q	A	127	G	D	A
21	A	A	Q	131	T	S	S
22	Q	Q	R	132	W	F	F
27	A	A	I	143	M	I	M
28	S	S	Y	149	Y	Y	V
29	H	S	S	152	R	R	S
32	V	V	L	153	N	N	G
35	L	L	F	155	A	G	S
37	I	V	I	156	V	V	I
41	I	A	I	161	L	L	F
42	L	I	L	168	W	W	F
45	T	M	T	169	I	I	V
47	G	R	G	179	I	I	M
48	P	P	E	181	L	L	F
51	P	L	D	182	K	N	N
52	K	K	S	192	S	S	A
53	G	R	G	196	I	I	T

54	Q	Q	K		204	I	I	L
57	M	L	L		207	K	K	W
58	K	K	R		209	R	R	K
60	V	L	L		210	N	N	D
62	T	T	K		211	I	V	I
63	N	A	W		216	Q	Q	K
66	L	F	L		218	G	G	P
67	L	L	F		222	F	F	L
70	I	I	V		223	G	A	A
71	Y	Y	F		225	F	I	I
73	L	F	L		229	F	W	W
74	G	G	V		236	C	L	L
80	A	A	G		239	L	L	I
83	M	L	I		242	Y	Y	F
84	Y	S	Y		244	Q	Q	K
85	T	V	T		245	T	T	S
89	M	L	Y		247	I	I	V
94	E	E	D		250	K	P	K
98	D	N	D		254	A	R	A
101	V	V	L		257	I	K	K
104	I	I	F		258	Q	E	V

* Position is based on alignment against EgD9e (SEQ ID NO:8), which has an identical sequence to that of EgD9eS (SEQ ID NO:10).

Of the 86 positions identified above in Table 11, those sites having greatest proximity to the periplasmic space, based on the membrane topology model of FIG. 4A, were eliminated from further consideration (i.e., positions 45, 47, 48, 51, 52, 53, 54, 57, 58, 60, 62, 63, 66, 67, 70, 71, 73, 74, 204, 207, 209, 210, 211, 216, 218, 222, 223, 225 and 229). Those sites highlighted in gray with bold text (i.e., positions 3, 5, 9, 12, 21, 22, 27, 28, 32, 37, 41, 42, 80, 84, 85, 94, 98, 101, 104, 105, 107, 108, 111, 115, 127, 131, 132, 143, 149, 152, 153, 156, 161, 168, 169, 179, 181, 182, 192, 196, 236, 239, 242, 244, 245, 247, 250, 254, 257 and 258 of EgD9eS) were selected for further experimental evaluation.

Slonomics® To Create SlonoMax® Mutant Genes Of EgD9eS-L35G

Slonomics® (U.S. Pat. 7,695,906) uses a set of double stranded DNA triplets as universal building blocks for the synthesis of combinatorial libraries “one codon at a time” (Sloning BioTechnology, Puchheim, Germany). For

library production, multiple codons can be introduced in parallel at any desired sequence position. The absence of functional bias and the ability to select and precisely control delivery of up to 20 codons at any ratio results in exceptionally high quality libraries containing the complete set of desired mutants.

SlonoMax® gene libraries (50 total) were thus created by Sloning BioTechnology, each gene library possessing at least 16 independent and unique sequence mutations at the targeted position (i.e., position 3, 5, 9, 12, 21, 22, 27, 28, 32, 37, 41, 42, 80, 84, 85, 94, 98, 101, 104, 105, 107, 108, 111, 115, 127, 131, 132, 143, 149, 152, 153, 156, 161, 168, 169, 179, 181, 182, 192, 196, 236, 239, 242, 244, 245, 247, 250, 254, 257 or 258 of EgD9eS), using pZuFmEgD9ES-L35G (SEQ ID NO:60) as the template.

All EgD9eS-L35G mutants were cloned into the vector backbone provided by pZuFmEgD9ES-L35G and subsequently transformed into *Y. lipolytica* strain Y2224 and cultured, as described in Example 2. The transformed cells (provided as frozen glycerol stocks) and DNA were obtained from Sloning BioTechnology. A small portion of transformed cells and DNA were sequenced and confirmed.

EXAMPLE 8

Identification of EgD9eS-L35G SlonoMax® Library Mutants Having Improved Delta-9 Elongase Conversion Efficiency

The present Example describes the identification of EgD9eS-L35G SlonoMax® mutants having improved delta-9 elongase conversion efficiency of LA to EDA, as compared to that of the variant protein EgD9eS-L35G identified in Example 6 (SEQ ID NO:59).

The fatty acid profiles of 807 *Yarrowia* transformants with constructs from the SlonoMax® library were screened using the “confirmation assay” methodology of Example 2, such that cells grown on fresh re-streaked MM plates were used to individually inoculate triplicate cultures comprising 3 mL liquid MM. In addition to the 807 mutants, *Yarrowia* strain Y2224

transformants, comprising pZuFmEgD9ES-L35G (SEQ ID NO:60) were inoculated in triplicate as experimental controls.

Data from selected mutants in the confirmation assay is presented in Table 12, and the FAME profiles of three representative EgD9eS-L35G controls are compared with those of the SlonoMax® library mutants demonstrating an increase in average % Conv. of LA to EDA. More specifically, the average (indicated with "Avg") concentration of each fatty acid as an area percent of TFAs ["% TFAs"] and the average % Conv. of LA to EDA (determined as described in Example 2) for each strain is shown below in Table 12. Fatty acids are identified based on the abbreviations of Example 4. Each strain description is indicative of the particular amino acid substitutions present in the respectively mutant EgD9eS gene. Thus, strain EgD9eS-L35G/S9A comprises a mutant pZuFmEgD9ES plasmid comprising a mutant EgD9eS gene, the gene having a L35G mutation and a S9A mutation when compared to the sequence of EgD9eS set forth as SEQ ID NO:10.

Table 12. Confirmation Assay: Lipid Composition In Transformant Y.
lipolytica Strain Y2224, Expressing EgD9eS-L35G Or SlonoMax® Mutant
Variants Thereof

Strain	Replicate No.	% TFAs						% Conv LA to EDA
		16:0	16:1	18:0	18:1	18:2	20:2	
EgD9eS-L35G Control-1	1	13.7	12.0	2.9	40.4	19.0	4.8	20.2
	2	14.2	12.7	2.9	41.7	19.5	4.4	18.5
	3	13.8	12.2	3.0	41.1	19.1	4.9	20.4
EgD9eS-L35G Control-2	1	13.8	12.5	2.8	40.7	19.8	4.5	18.4
	2	14.0	12.5	2.8	41.1	19.8	3.7	15.6
	3	13.8	12.3	2.9	41.0	19.7	4.5	18.6
EgD9eS-L35G Control-3	1	13.9	12.8	2.7	41.7	18.5	3.9	17.5
	2	13.7	12.7	2.7	42.6	19.0	3.7	16.2
	3	14.1	12.9	2.8	41.7	19.1	4.1	17.5
Avg. Control	--	13.9	12.5	2.8	41.3	19.3	4.3	18.1
EgD9eS-L35G/S9A	Avg of 3	12.3	12.8	3.1	49.9	12.9	3.8	22.8

EgD9eS-L35G/S9D	Avg of 3	12.3	12.3	3.2	48.6	12.2	4.3	25.6
EgD9eS-L35G/S9G	Avg of 3	12.6	12.7	3.1	51.1	12.2	3.8	23.3
EgD9eS-L35G/S9I	Avg of 3	13.0	12.2	2.9	52.5	12.2	3.1	20.4
EgD9eS-L35G/S9K	Avg of 3	12.4	12.4	2.9	52.3	12.1	3.5	22.1
EgD9eS-L35G/S9Q	Avg of 3	12.5	13.1	2.8	52.0	12.4	3.1	20.1
EgD9eS-L35G/Q12K	Avg of 3	12.5	14.1	2.6	51.4	11.6	3.3	22.3
EgD9eS-L35G/A21D	Avg of 3	12.4	14.2	2.7	49.7	12.1	3.3	21.4
EgD9eS-L35G/A21T	Avg of 3	12.3	13.3	2.9	50.3	13.2	3.3	20.0
EgD9eS-L35G/A21V	Avg of 3	12.7	15.1	2.3	49.1	13.4	3.6	21.3
EgD9eS-L35G/V32F	Avg of 3	12.2	14.9	2.4	49.4	14.0	3.2	18.8
EgD9eS-L35G/Y84C	Avg of 3	11.9	13.3	2.6	51.5	12.8	4.5	26.1
EgD9eS-L35G/L108G	Avg of 3	13.0	13.4	3.0	48.4	14.8	3.4	18.8
EgD9eS-L35G/G127L	Avg of 3	9.7	11.0	1.9	36.6	24.5	5.7	18.8
EgD9eS-L35G/W132T	Avg of 3	13.8	12.8	3.0	43.7	18.2	4.0	18.1
EgD9eS-L35G/M143N	Avg of 3	10.1	11.1	2.0	39.6	21.4	4.5	17.4
EgD9eS-L35G/M143W	Avg of 3	11.4	12.2	2.3	43.8	18.4	4.4	19.1
EgD9eS-L35G/L161T	Avg of 3	11.1	12.1	2.7	41.3	17.8	5.6	23.7
EgD9eS-L35G/L161Y	Avg of 3	9.9	11.8	2.6	37.4	22.3	6.1	21.5
EgD9eS-L35G/W168G	Avg of 3	11.5	12.3	2.5	44.0	17.6	4.7	20.8
EgD9eS-L35G/I179M	Avg of 3	13.8	12.5	3.0	41.5	18.5	4.3	18.8
EgD9eS-L35G/I179R	Avg of 3	10.2	11.9	2.2	40.5	18.4	6.3	25.5
EgD9eS-L35G/C236N	Avg of 3	13.3	13.4	2.8	45.5	16.9	3.8	18.5
EgD9eS-L35G/Q244N	Avg of 3	10.2	12.4	2.2	38.2	17.9	5.7	24.2
EgD9eS-L35G/A254W	Avg of 3	11.7	16.8	2.0	48.8	14.8	3.7	20.2
EgD9eS-L35G/A254Y	Avg of 3	13.1	16.2	2.5	48.4	12.9	3.4	21.0

It is noteworthy that the fatty acid profile and the % Conv. of LA to EDA of the replicate EgD9eS-L35G controls presented in Table 12 are somewhat different from the EgD9eS-L35G profiles previously presented. In the present set of experiments, the EgD9eS-L35G control “underperformed” in comparison to previous analyses (i.e., the average % Conv. of LA to EDA was determined to be about 18.1%, *supra*, while the average % Conv. of LA to EDA was determined to be about 26.6% and 27.2% in Example 6, Table

9). However, the transformants with EgD9eS-L35G produced 4.3 EDA % TFAs (average, *supra*), which was significantly greater than that produced in transformants with EgD9eS (i.e., 3.1 EDA % TFAs [Example 4, Table 5], 2.9 EDA % TFAs [Example 4, Table 6], and 3.5 EDA % TFAs [Example 6, Table 9]). For this reason, performance from previous experiments that repeated the functional analysis of EgD9eS-L35G (data not shown) was used in addition to EgD9eS-L35G performance in the present experiment as the basis for comparison of mutants from the EgD9eS site-evaluation library presented in Table 12.

Among the 26 selected elongase variants presented in Table 12, eleven were identified (highlighted in bold text) as demonstrating comparable or improved delta-9 elongase conversion activity relative to the control data of Table 12. These mutants included EgD9eS-L35G/S9D (141%), EgD9eS-L35G/A21V (118%), EgD9eS-L35G/V32F (104%), EgD9eS-L35G/Y84C (144%), EgD9eS-L35G/L108G (104%), EgD9eS-L35G/W132T (100%), EgD9eS-L35G/M143N (96%), EgD9eS-L35G/L161T (131%), EgD9eS-L35G/I179R (141%), EgD9eS-L35G/C236N (102%) and EgD9eS-L35G/Q244N (134%), wherein the delta-9 elongase conversion activity with respect to EgD9eS is shown in parentheses. Thus, up to a 44% improvement in LA to EDA conversion efficiency was demonstrated.

EXAMPLE 9

Creation Of A EgD9eS-L35G/ S9D/ A21V/ V32F/ Y84C/ L108G/ W132T/ M143N/ L161T/ I179R/ C236N/ Q244N Combinatorial Library

The present example describes the synthesis of a mutant EgD9eS combinatorial library, wherein various combinations of the beneficial mutations identified above in Example 8 (i.e., S9D, A21V, V32F, Y84C, L108G, W132T, M143N, L161T, I179R, C236N and Q244N) were "stacked" together into the EgD9eS mutant comprising the L35G mutation.

Creation Of Synthetic Primers For Construction Of The Combinatorial Library

Eleven pairs of primers were commercially synthesized, as described in SEQ ID NOs:64-85 (see Table 13, *infra*). Each primer pair was designed

to introduce one of the following mutations into the EgD9eS-L35G gene: S9D, A21V, V32F, Y84C, L108G, W132T, M143N, L161T, I179R, C236N and Q244N.

The primers were phosphorylated at 37 °C for 60 min using T4 polynucleotide kinase ["PNK"] (Cat. No. 70031Z, USB Corp.) and then deactivated at 65 °C for 20 min. Each 20 µl phosphorylation reaction mixture contained: 2.0 µl of 10x T4 PNK buffer, 15.0 µl of primer DNA (about 7 µM), 0.6 µl of 100 mM ATP, 0.4 µl of T4 PNK and 2.0 µl of water.

Multiple Mutation Site Mutagenesis To Create Combinatorial Mutant Genes Of EgD9eS-L35G

The Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit (Cat. No. 78480, USB Corporation, Cleveland, OH) was used to introduce the S9D, A21V, V32F, Y84C, L108G, W132T, M143N, L161T, I179R, C236N and Q244N mutations into EgD9eS-L35G in a series of 6 reactions, each reaction (with the exception of the final reaction) introducing two new mutations based on inclusion of a forward primer and reverse primer of Primer Set "A" and a forward primer and reverse primer of Primer Set "B" (Table 13). While the initial template in the series of reactions was EgD9eS-L35G, the product of Change-IT™ Rxn. 1 served as the template in Change-IT™ Rxn. 2, etc.

CL4783WOPCT

Table 13. Summary Of Change-IT™ Multiple Mutation Site Directed Mutagenesis Reactions

Change-IT™ Rxn. No.	Template	Product	Primer Set "A"		Primer Set "B"	
			Forward Primer	Reverse Primer	Forward Primer	Reverse Primer
#1	EgD9eS-L35G	Change-IT™ Rxn. #1	Eg_9D_122709f (SEQ ID NO:64)	Eg_84C_122709r (SEQ ID NO:65)	Eg_84C_122709f (SEQ ID NO:66)	Eg_9D_122709r (SEQ ID NO:67)
#2	Change-IT™ Rxn. #1	Change-IT™ Rxn. #2	Eg_161T_122709f (SEQ ID NO:68)	Eg_179R_122709r (SEQ ID NO:69)	Eg_179R_122709f (SEQ ID NO:70)	Eg_161T_122709r (SEQ ID NO:71)
#3	Change-IT™ Rxn. #2	Change-IT™ Rxn. #3	Eg_244N_122709f (SEQ ID NO:72)	Eg_21V_010710r (SEQ ID NO:73)	Eg_21V_010710f (SEQ ID NO:74)	Eg_244N_122709r (SEQ ID NO:75)
#4	Change-IT™ Rxn. #3	Change-IT™ Rxn. #4	Eg_32F_010710f (SEQ ID NO:76)	Eg_108G_010710r (SEQ ID NO:77)	Eg_108G_010710f (SEQ ID NO:78)	Eg_32F_010710r (SEQ ID NO:79)
#5	Change-IT™ Rxn. #4	Change-IT™ Rxn. #5	Eg_132T_010710f (SEQ ID NO:80)	Eg_143N_010710r (SEQ ID NO:81)	Eg_143N_010710f (SEQ ID NO:82)	Eg_132T_010710r (SEQ ID NO:83)
#6	Change-IT™ Rxn. #5	Change-IT™ Rxn. #6	Eg_236N_010710f (SEQ ID NO:84)	Eg_236N_010710r (SEQ ID NO:85)	--	--

More specifically, two 25 μ l PCR reaction mixtures were prepared, each one comprising 2.5 μ l of 10x Change-IT™ buffer, 2.5 μ l of phosphorylated forward primer, 2.5 μ l of phosphorylated reverse primer, 1.0 μ l of template (50 ng/ μ l), 15.5 μ l Nuclease-free water and 1.0 μ l Change-IT™ FidelityTaq enzyme. The first reaction utilized primers from primer set “A”, while the second utilized primer set “B” primers. PCR amplification was performed using the following conditions: 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, and elongation/ligation at 68 °C for 25 min. A final elongation/ligation cycle at 68 °C for 30 min was carried out, followed by the reaction termination at 4 °C.

Following amplification, the template was removed by adding *DpnI* enzyme and digestion was performed at 37 °C for 3 hr. The PCR DNA was used to transform *E. coli* Top 10 electro-competent cells (Cat. No. C404052, Invitrogen, Carlsbad, CA) by electroporation. The transformed cells were spread onto LB with 100 mg/L ampicillin agar plates and grown in a 37 °C incubator overnight. Plasmid DNA was extracted from the transformant *E. coli* cells using a QIAprep® Spin Miniprep kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocol. The purified DNA was then used as template in the next Change-IT™ reaction. Following the sixth reaction, which introduced the last of the 11 mutations into the original EgD9eS-L35G template, DNA was purified from the transformant *E. coli* cells, as described above. The DNA was then transformed into *Y. lipolytica* strain Y2224 (*supra*, Example 2).

EXAMPLE 10

Identification of EgD9eS-L35G/ S9D/ A21V/ V32F/ Y84C/ L108G/ W132T/ M143N/ L161T/ I179R/ C236N/ Q244N Combinatorial Library Mutants Having Improved Delta-9 Elongase Conversion Efficiency

The present Example describes: 1) the identification of EgD9eS-L35G/ S9D/ A21V/ V32F/ Y84C/ L108G/ W132T/ M143N/ L161T/ I179R/ C236N/ Q244N combinatorial library mutants having improved delta-9 elongase conversion efficiency of LA to EDA, as compared to that of the wildtype protein EgD9eS (SEQ ID NO:10); 2) sequence analysis of these EgD9eS mutants; and,

3) re-creation of the sequenced EgD9eS mutants to confirm the improved delta-9 elongase conversion efficiency.

The fatty acid profiles of 2,388 *Yarrowia* transformants with constructs from the combinatorial library (Example 9) were screened using the quick screen “plate assay” of Example 2. Most of these mutants exhibited reduced conversion of LA to EDA compared to the wild type control, EgD9eS (SEQ ID NO:10). However, five transformants were confirmed to exhibit improved delta-9 elongation activity as compared to the control, based on confirmation assays of Example 2.

The DNA sequences of the mutant EgD9eS genes were determined using colony PCR. In brief, a small quantity of yeast cells was sampled from freshly streaked plates using a sterile pipette tip and the cells were suspended in 20 μ l of molecular grade water. Cell suspension (2 μ l) was transferred to TaKaRa Ex Taq PCR mix prepared according to the manufacturer's recommendation (Takara Biotechnology Co., LTD.). The primers used for colony PCR were forward primer FBAIN-F (SEQ ID NO:98) and reverse primer Y1026 (SEQ ID NO:99). The thermal cycler program included an initial denaturation of template at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 sec, annealing at 56 °C for 30 sec and extension at 72 °C for 3 min. A final extension at 72 °C for 6 min was carried out.

The PCR products were sequenced with primers FBAIN-F (SEQ ID NO:98) and Y1026 (SEQ ID NO:99). Analysis of the DNA sequence data revealed the nucleotide substitutions and expressed amino acid substitutions within the mutant EgD9eS genes. A designation indicative of the amino acid substitution was given to the mutant EgD9eS gene and to the mutant pZuFmEgD9ES plasmid comprising the mutant EgD9eS gene, as shown in Table 14.

Table 14. Summary of Sequenced EgD9eS Combinatorial Library Mutants

Combinatorial Mutant	Nucleotide Substitution	Resulting Amino Acid Substitution	Designation For Mutant Gene	Designation For Mutant Plasmid
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EgD9EN-427	C103G, T104G, and C105G	L35G	EgD9eS-L35G/W132T/I179R (SEQ ID NO:100)	pZuFmEgD9ES-L35G/W132T/I179R (SEQ ID NO:102)
	T394A, G395C, G396C	W132T		
	A535C, T536G, C537A	I179R		
EgD9EN-1043	T25G, C26A	S9D	EgD9eS-S9D/L35G/Y84C/I179R (SEQ ID NO:103)	pZuFmEgD9ES-S9D/L35G/Y84C/I179R (SEQ ID NO:105)
	C103G, T104G, and C105G	L35G		
	A251G, C252T	Y84C		
	A535C, T536G, C537A	I179R		
EgD9EN-1534	C62T and T63G	A21V	EgD9eS-A21V/L35G/L108G/I179R (SEQ ID NO:86)	pZuFmEgD9ES-A21V/L35G/L108G/I179R (SEQ ID NO:88)
	C103G, T104G and C105G	L35G		
	C322G, T323G and G324T	L108G		
	A535C, T536G and C537A	I179R		
EgD9EN-1635	C103G, T104G, and C105G	L35G	EgD9eS-L35G/Y84C/I179R/Q244N (SEQ ID NO:106)	pZuFmEgD9ES-L35G/Y84C/I179R/Q244N (SEQ ID NO:108)
	A251G, C252T	Y84C		
	A535C, T536G, C537A	I179R		
	C730A, G732C	Q244N		
EgD9EN-1734	C62T, T63G	A21V	EgD9eS-A21V/L35G/W132T/I179R/Q244N (SEQ ID NO:109)	pZuFmEgD9ES-A21V/L35G/W132T/I179R/Q244N (SEQ ID NO:111)
	C103G, T104G, and C105G	L35G		
	T394A, G395C, G396C	W132T		
	A535C, T536G, C537A	I179R		
	C730A, G732C	Q244N		

New primers for site-directed mutagenesis were designed, based on the amino acid substitutions of Table 14. These primers were then utilized in reactions designed to introduce the EgD9eS mutations within the “megaprimers” into pZuFmEgD9ES (FIG. 2; SEQ ID NO:25), thereby replacing the non-mutant EgD9eS gene with the various mutant EgD9eS genes listed in Table 14.

This was accomplished using the QuikChange® II XL site directed mutagenesis kit (Cat. No. 200524, Stratagene, La Jolla, CA), as described in Example 3.

These mutant genes were transformed into *E. coli* Top 10 electro-competent cells, purified, sequenced, and subsequently transformed into *Y. lipolytica* strain Y2224, as described in Example 2. In this way, the mutant EgD9eS genes shown in Table 14 were recreated on plasmids and re-introduced back into strain Y2224 to confirm that the improved delta-9 elongase conversion efficiency exhibited by the EgD9eS combinatorial mutants was attributed to the identified amino acid substitutions.

Data from these confirmation assays are presented in Table 15, and the FAME profiles of individual pZuFmEgD9ES control transformants are compared with those mutants of the combinatorial library. For a conservative comparison, the data shown for each strain represents the FAME profiles for the 3 isolates with highest % Conv. of LA to EDA for each strain. More specifically, the concentration of each fatty acid as an area percent of TFAs [% TFAs] and % Conv. of LA to EDA (determined as described in Example 2) for each strain is shown below, while averages are highlighted in gray and indicated with “Avg”. Fatty acids are identified based on the abbreviations of Example 4.

Table 15. Confirmation Assay: Lipid Composition In Transformant *Y. lipolytica* Strain Y2224, Expressing EgD9eS Or Combinatorial Mutant Variants Thereof

Strain	Replicate No.	% TFAs						%Conv LA to EDA
		16:0	16:1	18:0	18:1	18:2	20:2	
EgD9eS- Control	1	12.5	12.6	2.8	50.6	13.1	2.6	16.7
	2	12.1	13.1	2.5	52.1	13.4	2.5	15.8
	3	12.5	13.1	2.8	51.0	13.3	2.5	15.8

	Avg	12.4	12.9	2.7	51.2	13.2	2.5	16.1
EgD9EN-427	1	11.6	13.2	2.6	49.7	14.2	3.2	18.1
	2	12.2	12.7	2.6	51.5	13.0	2.9	18.0
	3	11.8	12.7	2.5	52.4	13.0	2.7	17.2
	Avg	11.9	12.9	2.6	51.2	13.4	2.9	17.8
EgD9EN-1043	1	11.7	13.3	2.4	52.2	13.3	2.8	17.7
	2	11.8	12.8	2.5	51.9	12.8	2.8	17.9
	3	11.8	12.6	2.5	51.6	12.8	2.6	16.9
	Avg	11.8	12.9	2.4	51.9	13.0	2.8	17.5
EgD9EN-1534	1	11.4	11.8	2.4	48.4	12.5	2.6	17.2
	2	12.0	12.4	2.5	49.8	13.3	2.6	16.6
	3	12.2	12.4	2.6	50.8	13.1	2.6	16.6
	Avg	11.9	12.2	2.5	49.7	13.0	2.6	16.8
EgD9EN-1635	1	11.1	12.9	2.4	51.2	13.1	3.0	18.8
	2	11.5	13.8	2.5	49.4	14.1	3.1	18.1
	3	11.9	13.5	2.6	50.1	14.1	2.9	17.1
	Avg	11.5	13.4	2.5	50.2	13.8	3.0	18.0
EgD9EN-1734	1	11.5	12.7	2.4	51.7	12.4	3.3	21.2
	2	11.3	12.5	2.2	51.3	12.1	3.2	20.7
	3	11.8	12.8	2.4	52.8	13.0	2.8	18.0
	Avg	11.5	12.7	2.3	51.9	12.5	3.1	20.0

Clones of *Y. lipolytica* strain Y2224 that were transformed with pZuFmEgD9ES, comprising the codon-optimized EgD9eS gene of SEQ ID NO:10 (non-mutant), produced an average of 2.5 EDA % TFAs, wherein the average conversion efficiency [% Conv] of LA to EDA in these three clones was determined to be about 16.1%. In contrast, the average % Conv of LA to EDA for mutant strain EgD9EN-427 was 17.8% (or 110% relative to the control). Similarly, the average % Conv of LA to EDA for mutant strain EgD9EN-1043 was 17.5% (or 108% relative to the control). The average % Conv of LA to EDA for mutant strain EgD9EN-1534 was 16.8% (or 104% relative to the control); the average % Conv for mutant strain EgD9EN-1635 was 18.0% (or 111% relative to the control); and, the average % Conv for mutant strain EgD9EN-1734 was 20.0% (or 123% relative to the control).

Thus, these experiments thereby confirmed the improved delta-9 elongase conversion efficiency exhibited by EgD9eS combinatorial library mutants

EgD9EN-427, EgD9EN-1043, EgD9EN-1534, EgD9EN-1635, and EgD9EN-1734, wherein the improvement ranged from 4-23%.

EXAMPLE 11

Generation Of *Yarrowia lipolytica* Strain Z1978 To Produce About 58.7% EPA Of Total Fatty Acids

The present Example describes the construction of strain Z1978, derived from *Y. lipolytica* ATCC #20362, capable of producing about 58.7% EPA relative to the total lipids with 38.3% total lipid content ["TFAs % DCW"] via expression of a delta-9 elongase/ delta-8 desaturase pathway. This strain includes the delta-9 elongase site-saturation mutant of Examples 5 and 6, comprising the L35G mutation (i.e., EgD9eS-L35G [SEQ ID NOs:58 and 59]).

The development of strain Z1978 (FIG. 6) required the construction of strains Y2224, Y4001, Y4001U, Y4036, Y4036U, L135, L135U9, Y8002, Y8006U6, Y8069, Y8069U, Y8154, Y8154U, Y8269, Y8269U, Y8412U6, Y8647, Y8467U, Y9028, Y9028U, Y9502 and strain Y9502U.

Fatty Acid Analysis Of *Yarrowia lipolytica* During Strain Constructions

For fatty acid ["FA"] analysis, cells were collected by centrifugation and lipids were extracted as described in Bligh, E. G. & Dyer, W. J. (*Can. J. Biochem. Physiol.*, 37:911-917 (1959)). Fatty acid methyl esters ["FAMES"] were prepared by transesterification of the lipid extract with sodium methoxide (Roughan, G., and Nishida I., *Arch Biochem Biophys.*, 276(1):38-46 (1990)) and subsequently analyzed with a Hewlett-Packard 6890 GC fitted with a 30-m X 0.25 mm (i.d.) HP-INNOWAX (Hewlett-Packard) column. The oven temperature was from 170 °C (25 min hold) to 185 °C at 3.5 °C/min.

For direct base transesterification, *Yarrowia* cells (0.5 mL culture) were harvested, washed once in distilled water, and dried under vacuum in a Speed-Vac for 5-10 min. Sodium methoxide (100 µL of 1%) and a known amount of C15:0 triacylglycerol (C15:0 TAG; Cat. No. T-145, Nu-Check Prep, Elysian, MN) was added to the sample, and then the sample was vortexed and rocked for 30 min at 50 °C. After adding 3 drops of 1 M NaCl and 400 µL hexane, the sample was vortexed and spun. The upper layer was removed and analyzed by GC.

Alternately, a modification of the base-catalysed transesterification method described in *Lipid Analysis*, William W. Christie, 2003 was used for routine analysis of the broth samples from either fermentation or flask samples. Specifically, broth samples were rapidly thawed in room temperature water, then weighed (to 0.1 mg) into a tarred 2 mL microcentrifuge tube with a 0.22 µm Corning® Costar® Spin-X® centrifuge tube filter (Cat. No. 8161). Sample (75 - 800 µl) was used, depending on the previously determined DCW. Using an Eppendorf 5430 centrifuge, samples are centrifuged for 5-7 min at 14,000 rpm or as long as necessary to remove the broth. The filter was removed, liquid was drained, and ~500 µl of deionized water was added to the filter to wash the sample. After centrifugation to remove the water, the filter was again removed, the liquid drained and the filter re-inserted. The tube was then re-inserted into the centrifuge, this time with the top open, for ~3-5 min to dry. The filter was then cut approximately ½ way up the tube and inserted into a fresh 2 mL round bottom Eppendorf tube (Cat. No. 22 36 335-2).

The filter was pressed to the bottom of the tube with an appropriate tool that only touches the rim of the cut filter container and not the sample or filter material. A known amount of C15:0 TAG (*supra*) in toluene was added and 500 µl of freshly made 1% sodium methoxide in methanol solution. The sample pellet was firmly broken up with the appropriate tool and the tubes were closed and placed in a 50 °C heat block (VWR Cat. No. 12621-088) for 30 min. The tubes were then allowed to cool for at least 5 min. Then, 400 µl of hexane and 500 µl of a 1 M NaCl in water solution were added, the tubes were vortexed for 2x 6 sec and centrifuged for 1 min. Approximately 150 µl of the top (organic) layer was placed into a GC vial with an insert and analyzed by GC.

FAME peaks recorded via GC analysis were identified by their retention times, when compared to that of known fatty acids, and quantitated by comparing the FAME peak areas with that of the internal standard (C15:0 TAG) of known amount. Thus, the approximate amount (µg) of any fatty acid FAME [“µg FAME”] is calculated according to the formula: (area of the FAME peak for the specified fatty acid/ area of the standard FAME peak) * (µg of the standard C15:0 TAG),

while the amount (μg) of any fatty acid [$\mu\text{g FA}$] is calculated according to the formula: (area of the FAME peak for the specified fatty acid/area of the standard FAME peak) * (μg of the standard C15:0 TAG) * 0.9503, since 1 μg of C15:0 TAG is equal to 0.9503 μg fatty acids. Note that the 0.9503 conversion factor is an approximation of the value determined for most fatty acids, which range between 0.95 and 0.96.

The lipid profile, summarizing the amount of each individual fatty acid as a weight percent of TFAs, was determined by dividing the individual FAME peak area by the sum of all FAME peak areas and multiplying by 100.

Analysis Of Total Lipid Content And Composition In *Yarrowia lipolytica* During Strain Constructions By Flask Assay

For a detailed analysis of the total lipid content and composition in a particular strain of *Y. lipolytica*, flask assays were conducted as followed. Specifically, one loop of freshly streaked cells was inoculated into 3 mL FM medium and grown overnight at 250 rpm and 30 °C. The OD_{600nm} was measured and an aliquot of the cells were added to a final OD_{600nm} of 0.3 in 25 mL FM medium in a 125 mL flask. After 2 days in a shaker incubator at 250 rpm and at 30 °C, 6 mL of the culture was harvested by centrifugation and resuspended in 25 mL HGM in a 125 mL flask. After 5 days in a shaker incubator at 250 rpm and at 30 °C, a 1 mL aliquot was used for fatty acid analysis (*supra*) and 10 mL dried for dry cell weight ["DCW"] determination.

For DCW determination, 10 mL culture was harvested by centrifugation for 5 min at 4000 rpm in a Beckman GH-3.8 rotor in a Beckman GS-6R centrifuge. The pellet was resuspended in 25 mL of water and re-harvested as above. The washed pellet was re-suspended in 20 mL of water and transferred to a pre-weighed aluminum pan. The cell suspension was dried overnight in a vacuum oven at 80 °C. The weight of the cells was determined.

Total lipid content of cells ["TFAs % DCW"] is calculated and considered in conjunction with data tabulating the concentration of each fatty acid as a weight percent of TFAs ["% TFAs"] and the EPA content as a percent of the dry cell weight ["EPA % DCW"]. Data from flask assays are presented as a table that

summarizes the total dry cell weight of the cells ["DCW"], the total lipid content of cells ["TFAs % DCW"], the concentration of each fatty acid as a weight percent of TFAs ["% TFAs"] and the EPA content as a percent of the dry cell weight ["EPA % DCW"]. More specifically, fatty acids are identified as 16:0 (palmitate), 16:1 (palmitoleic acid), 18:0 (stearic acid), 18:1 (oleic acid), 18:2 (LA), ALA, EDA, DGLA, ARA, ETrA, ETA, EPA and other.

Genotype Of *Yarrowia lipolytica* Strain Y9502

The generation of strain Y9502 is described in U.S. Pat. Pub. No. 2010-0317072-A1. Strain Y9502, derived from *Y. lipolytica* ATCC #20362, was capable of producing about 57.0% EPA relative to the total lipids via expression of a delta-9 elongase/delta-8 desaturase pathway (FIG. 6).

The final genotype of strain Y9502 with respect to wildtype *Yarrowia lipolytica* ATCC #20362 was *Ura*⁺, *Pex3*⁻, *unknown 1*⁻, *unknown 2*⁻, *unknown 3*⁻, *unknown 4*⁻, *unknown 5*⁻, *unknown6*⁻, *unknown 7*⁻, *unknown 8*⁻, *unknown9*⁻, *unknown 10*⁻, YAT1::ME3S::Pex16, GPD::ME3S::Pex20, YAT1::ME3S::Lip1, FBAINm::EgD9eS::Lip2, EXP1::EgD9eS::Lip1, GPAT::EgD9e::Lip2, YAT1::EgD9eS::Lip2, FBAINm::EgD8M::Pex20, EXP1::EgD8M::Pex16, FBAIN::EgD8M::Lip1, GPD::EaD8S::Pex16 (2 copies), YAT1::E389D9eS/EgD8M::Lip1, YAT1::EgD9eS/EgD8M::Aco, FBAINm::EaD9eS/EaD8S::Lip2, GPD::FmD12::Pex20, YAT1::FmD12::Oct, EXP1::FmD12S::Aco, GPDIN::FmD12::Pex16, EXP1::EgD5M::Pex16, FBAIN::EgD5SM::Pex20, GPDIN::EgD5SM::Aco, GPM::EgD5SM::Oct, EXP1::EgD5SM::Lip1, YAT1::EaD5SM::Oct, FBAINm::PaD17::Aco, EXP1::PaD17::Pex16, YAT1::PaD17S::Lip1, YAT1::YICPT1::Aco, YAT1::MCS::Lip1, FBA::MCS::Lip1, YAT1::MaLPAAT1S::Pex16. Abbreviations are as follows: FmD12 is a *Fusarium moniliforme* delta-12 desaturase gene [U.S. Pat. 7,504,259]; FmD12S is a codon-optimized delta-12 desaturase gene, derived from *F. moniliforme* [U.S. Pat. 7,504,259]; ME3S is a codon-optimized C_{16/18} elongase gene, derived from *Mortierella alpina* [U.S. Pat. 7,470,532]; EgD9e is a *Euglena gracilis* delta-9 elongase gene [U.S. Pat. 7,645,604]; EgD9eS is a codon-optimized delta-9 elongase gene, derived from *E. gracilis*

[U.S. Pat. 7,645,604]; EgD8M is a synthetic mutant delta-8 desaturase gene [U.S. Pat. 7,709,239], derived from *E. gracilis* [U.S. Pat. 7,256,033]; EaD8S is a codon-optimized delta-8 desaturase gene, derived from *Euglena anabaena* [U.S. Pat. 7,790,156]; E389D9eS/EgD8M is a DGLA synthase created by linking a codon-optimized delta-9 elongase gene ("E389D9eS"), derived from *Eutreptiella* sp. CCMP389 delta-9 elongase (U.S. Pat. 7,645,604) to the delta-8 desaturase "EgD8M" (*supra*) [U.S. Pat. Pub. No. 2008-0254191-A1]; EgD9eS/EgD8M is a DGLA synthase created by linking the delta-9 elongase "EgD9eS" (*supra*) to the delta-8 desaturase "EgD8M" (*supra*) [U.S. Pat. Pub. No. 2008-0254191-A1]; EaD9eS/EgD8M is a DGLA synthase created by linking a codon-optimized delta-9 elongase gene ("EaD9eS"), derived from *E. anabaena* delta-9 elongase [U.S. Pat. 7,794,701] to the delta-8 desaturase "EgD8M" (*supra*) [U.S. Pat. Pub. No. 2008-0254191-A1]; EgD5M and EgD5SM are synthetic mutant delta-5 desaturase genes [U.S. Pat. Pub. No. 2010-0075386-A1], derived from *E. gracilis* [U.S. Pat. 7,678,560]; EaD5SM is a synthetic mutant delta-5 desaturase gene [U.S. Pat. Pub. No. 2010-0075386-A1], derived from *E. anabaena* [U.S. Pat. 7,943,365]; PaD17 is a *Pythium aphanidermatum* delta-17 desaturase gene [U.S. Pat. 7,556,949]; PaD17S is a codon-optimized delta-17 desaturase gene, derived from *P. aphanidermatum* [U.S. Pat. 7,556,949]; YICPT1 is a *Yarrowia lipolytica* diacylglycerol cholinephosphotransferase gene [U.S. Pat. 7,932,077]; MCS is a codon-optimized malonyl-CoA synthetase gene, derived from *Rhizobium leguminosarum* bv. *viciae* 3841 [U.S. Pat. Pub. No. 2010-0159558-A1], and, MaLPAAT1S is a codon-optimized lysophosphatidic acid acyltransferase gene, derived from *Mortierella alpina* [U.S. Pat. 7,879,591].

For a detailed analysis of the total lipid content and composition in strain Y9502, a flask assay was conducted wherein cells were grown in 2 stages for a total of 7 days. Based on analyses, strain Y9502 produced 3.8 g/L DCW, 37.1 TFAs % DCW, 21.3 EPA % DCW, and the lipid profile was as follows, wherein the concentration of each fatty acid is as a weight percent of TFAs ["% TFAs"]: 16:0 (palmitate)—2.5, 16:1 (palmitoleic acid)—0.5, 18:0 (stearic acid)—2.9, 18:1

(oleic acid)-- 5.0, 18:2 (LA)—12.7, ALA—0.9, EDA—3.5, DGLA—3.3, ARA--0.8, ETrA--0.7, ETA—2.4, EPA—57.0, other—7.5.

Generation Of Strain Y9502U (*Ura3*-)

To disrupt the *Ura3* gene in strain Y9502, *Sall/PacI*-digested construct pZKUM (FIG. 7A; SEQ ID NO:89; described in Table 15 of U.S. Pat. Pub. No. 2009-0093543-A1, hereby incorporated herein by reference) was used to integrate an *Ura3* mutant gene into the *Ura3* gene of strain Y9502, according to the General Methods. A total of 27 transformants (selected from a first group comprising 8 transformants, a second group comprising 8 transformants, and a third group comprising 11 transformants) were grown on Minimal Media + 5-fluoroorotic acid ["MM+ 5-FOA"] selection plates and maintained at 30 °C for 2 to 5 days. Further experiments determined that only the third group of transformants possessed a real *Ura*- phenotype.

The *Ura*- cells were scraped from the MM + 5-FOA plates and subjected to fatty acid analysis, according to the General Methods. In this way, GC analyses showed that there were 28.5%, 28.5%, 27.4%, 28.6%, 29.2%, 30.3% and 29.6% EPA of TFAs in pZKUM-transformants #1, #3, #6, #7, #8, #10 and #11 grown on MM + 5-FOA plates of group 3, respectively. These seven strains were designated as strains Y9502U12, Y9502U14, Y9502U17, Y9502U18, Y9502U19, Y9502U21 and Y9502U22, respectively (collectively, Y9502U).

Generation Of Strain Z1978

Construct pZKL3-9DP9N (FIG. 7B; SEQ ID NO:90) was then generated to integrate one delta-9 desaturase gene, one choline-phosphate cytidylyl-transferase gene, and one delta-9 elongase mutant gene into the *Yarrowia* YALI0F32131p locus (GenBank Accession No. XM_506121) of strain Y9502U. The pZKL3-9DP9N plasmid contained the following components:

Table 16. Description of Plasmid pZKL3-9DP9N (SEQ ID NO:90)

RE Sites And Nucleotides Within SEQ ID NO:90	Description Of Fragment And Chimeric Gene Components
<i>AscI/BsiWI</i> (887-4)	884 bp 5' portion of YALI0F32131p locus (GenBank Accession No. XM_506121, labeled as "Lip3-5" in Figure)
<i>PacI/SphI</i> (4396-3596)	801 bp 3' portion of YALI0F32131p locus (GenBank Accession No. XM_506121, labeled as "Lip3-3" in Figure)
<i>SwaI/BsWI</i> (11716 - 1)	YAT1::EgD9eS-L35G::Pex20, comprising: • YAT1: <i>Yarrowia lipolytica</i> YAT1 promoter (labeled as "YAT" in Figure; U.S. Pat. Pub. No. 2010-0068789-A1); • EgD9eS-L35G: Synthetic mutant of delta-9 elongase gene (SEQ ID NO:58), derived from <i>Euglena gracilis</i> ("EgD9eS"; U.S. Pat. 7,645,604) (labeled as "EgD9ES-24" in Figure); • Pex20: Pex20 terminator sequence from <i>Yarrowia Pex20</i> gene (GenBank Accession No. AF054613)
<i>PmeI/SwaI</i> (8759-11716)	GPDIN::YID9::Lip1, comprising: • GPDIN: <i>Yarrowia lipolytica</i> GPDIN promoter (U.S. Pat. 7,459,546; labeled as "GPDPro+Intron" in Figure); • YID9: <i>Yarrowia lipolytica</i> delta-9 desaturase gene (GenBank Accession No. XM_501496; SEQ ID NO:91) (labeled as "YID9D" in Figure); • Lip1: Lip1 terminator sequence from <i>Yarrowia Lip1</i> gene (GenBank Accession No. Z50020)
<i>ClalI/PmeI</i> (6501-8759)	EXP1::YIPCT::Pex16, comprising: • EXP1: <i>Yarrowia lipolytica</i> export protein (EXP1) promoter (labeled as "EXP" in Figure; U.S. Pat. 7,932,077); • YIPCT: <i>Yarrowia lipolytica</i> choline-phosphate cytidylyl-transferase ["PCT"] gene (GenBank Accession No. XM_502978; SEQ ID NO:93); • Pex16: Pex16 terminator sequence from <i>Yarrowia Pex16</i> gene (GenBank Accession No. U75433)
<i>SalI/EcoRI</i> (6501-4432)	<i>Yarrowia Ura3</i> gene (GenBank Accession No. AJ306421)

The pZKL3-9DP9N plasmid was digested with *AscI/SphI*, and then used for transformation of strain Y9502U17. The transformant cells were plated onto Minimal Media ["MM"] plates and maintained at 30 °C for 3 to 4 days. Single colonies were re-streaked onto MM plates, and then inoculated into liquid MM at 30 °C and shaken at 250 rpm/min for 2 days. The cells were collected by

centrifugation, resuspended in High Glucose Media ["HGM"] and then shaken at 250 rpm/min for 5 days. The cells were subjected to fatty acid analysis, *supra*.

GC analyses showed that most of the selected 96 strains of Y9502U17 with pZKL3-9DP9N produced 50-56% EPA of TFAs. Five strains (i.e., #31, #32, #35, #70 and #80) that produced about 59.0%, 56.6%, 58.9%, 56.5%, and 57.6% EPA of TFAs were designated as strains Z1977, Z1978, Z1979, Z1980 and Z1981, respectively.

The final genotype of these pZKL3-9DP9N transformant strains with respect to wildtype *Yarrowia lipolytica* ATCC #20362 was *Ura+*, *Pex3-*, *unknown 1-*, *unknown 2-*, *unknown 3-*, *unknown 4-*, *unknown 5-*, *unknown6-*, *unknown 7-*, *unknown 8-*, *unknown9-*, *unknown 10-*, *unknown 11-*, YAT1::ME3S::Pex16, GPD::ME3S::Pex20, YAT1::ME3S::Lip1, FBAINm::EgD9eS::Lip2, EXP1::EgD9eS::Lip1, GPAT::EgD9e::Lip2, YAT1::EgD9eS::Lip2, YAT1::EgD9eS-L35G::Pex20, FBAINm::EgD8M::Pex20, EXP1::EgD8M::Pex16, FBAIN::EgD8M::Lip1, GPD::EaD8S::Pex16 (2 copies), YAT1::E389D9eS/EgD8M::Lip1, YAT1::EgD9eS/EgD8M::Aco, FBAINm::EaD9eS/EaD8S::Lip2, GPDIN::YID9::Lip1, GPD::FmD12::Pex20, YAT1::FmD12::Oct, EXP1::FmD12S::Aco, GPDIN::FmD12::Pex16, EXP1::EgD5M::Pex16, FBAIN::EgD5SM::Pex20, GPDIN::EgD5SM::Aco, GPM::EgD5SM::Oct, EXP1::EgD5SM::Lip1, YAT1::EaD5SM::Oct, FBAINm::PaD17::Aco, EXP1::PaD17::Pex16, YAT1::PaD17S::Lip1, YAT1::YICPT1::Aco, YAT1::MCS::Lip1, FBA::MCS::Lip1, YAT1::MaLPAAT1S::Pex16, EXP1::YIPCT::Pex16.

Knockout of the YALI0F32131p locus (GenBank Accession No. XM_50612) in strains Z1977, Z1978, Z1979, Z1980 and Z1981 was not confirmed in any of these EPA strains produced by transformation with pZKL3-9DP9N.

Cells from YPD plates of strains Z1977, Z1978, Z1979, Z1980 and Z1981 were grown and analyzed for total lipid content and composition by flask assays. Table 17 below summarizes total lipid content and composition in each of these strains. Specifically, the Table summarizes the total DCW, the TFAs % DCW,

the concentration of each fatty acid as a weight percent of TFAs [% TFAs] and the EPA % DCW.

CL4783WOPCT

Table 17. Total Lipid Content And Composition In *Yarrowia* Strains Z1977, Z1978, Z1979, Z1980 and Z1981 By Flask

Assay

Strain	DCW (g/L)	TFAs % DCW	% TFAs													EPA % DCW
			16:0	16:1	18:0	18:1	18:2	ALA	EDA	DGLA	ARA	EtrA	ETA	EPA	other	
Z1977	3.8	34.3	2.0	0.5	1.9	4.6	11.2	0.7	3.1	3.3	0.9	0.7	2.2	59.1	9.9	20.3
Z1978	3.9	38.3	2.4	0.4	2.4	4.8	11.1	0.7	3.2	3.3	0.8	0.6	2.1	58.7	9.5	22.5
Z1979	3.7	33.7	2.3	0.4	2.4	4.1	10.5	0.6	3.2	3.6	0.9	0.6	2.2	59.4	9.8	20.0
Z1980	3.6	32.7	2.1	0.4	2.2	4.0	10.8	0.6	3.1	3.5	0.9	0.7	2.2	59.5	10.0	19.5
Z1981	3.5	34.3	2.2	0.4	2.1	4.2	10.6	0.6	3.3	3.4	1.0	0.8	2.2	58.5	10.7	20.1

Strain Z1978 was subsequently subjected to partial genome sequencing. This work, as described in U.S. Provisional Application No. 61/428,277 (E.I. duPont de Nemours & Co., Inc., Attorney Docket Number CL5267USPRV, filed December 30, 2010), determined that instead of six delta-5 desaturase genes integrated into the *Yarrowia* genome (i.e., chimeric genes EXP1::EgD5M::Pex16, FBAIN::EgD5SM::Pex20, GPDIN::EgD5SM::Aco, GPM::EgD5SM::Oct, EXP1::EgD5SM::Lip1, YAT1::EaD5SM::Oct), the engineered strain actually possessed only four delta-5 desaturase genes (i.e., EXP1::EgD5M::Pex16, FBAIN::EgD5SM::Pex20, EXP1::EgD5SM::Lip1, and YAT1::EaD5SM::Oct).

Comparison Of *Yarrowia lipolytica* Strain Y9502 And Strain Z1978

The heterologous genes expressed in strain Z1978 differ from those expressed in strain Y9502 only by the additional expression of one delta-9 desaturase gene, one cholinephosphate cytidyltransferase gene, and one delta-9 elongase mutant (i.e., EgD9eS-L35G, as set forth in SEQ ID NOs:58 and 59). The total delta-9 elongase conversion efficiency [% Conv] of LA and ALA to EPA was calculated in Table 18 for Y9502 and Z1978 strains according to the following formula: $([\text{product}]/[\text{substrate} + \text{product}]) \times 100$, wherein the product was the sum of EDA % TFAs, ETrA % TFAs, DGLA % TFAs, ETA % TFAs, ARA % TFAs and EPA % TFAs and the substrate was the sum of LA % TFAs, ALA % TFAs, EDA % TFAs, ETrA % TFAs, DGLA % TFAs, ETA % TFAs, ARA % TFAs and EPA % TFAs.

Table 18. Comparison Of Total Lipid Content And Composition And Delta-9 Elongase Activity In Transformant *Y. lipolytica* Strains Y9502 And Z1978

		<i>Yarrowia lipolytica</i> strain Y9502	<i>Yarrowia lipolytica</i> strain Z1978
DCW (g/L)		3.8	3.9
TFAs % DCW		37.1	38.3
% TFAs	16:0	2.5	2.4
	16:1	0.5	0.4
	18:0	2.9	2.4
	18:1	5	4.8
	18:2	12.7	11.1
	ALA	0.9	0.7

	EDA	3.5	3.2
	DGLA	3.3	3.3
	ARA	0.8	0.8
	ETrA	0.7	0.6
	ETA	2.4	2.1
	EPA	57	58.7
	Other	7.5	9.5
EPA % DCW		21.3	22.5
Total %Conv LA to EPA		83.3	85.3

As shown above, the total delta-9 elongase conversion efficiency was determined to be 83.3% in strain Y9502, while the efficiency was improved in strain Z1978 (i.e., 85.3%). Based on this improvement in the delta-9 elongase activity, EgD9eS-L35G is considered a useful mutant gene to be used in a functional delta-9 elongase/ delta-8 desaturase pathway for biosynthesis of PUFAs.

Any of the mutant delta-9 elongases of the invention herein could be similarly introduced into an appropriate vector for expression in a preferred strain of *Yarrowia lipolytica*, as demonstrated in this example.

The claims defining the invention are as follows:

1. An isolated polynucleotide comprising:
 - (a) a nucleotide sequence encoding a mutant polypeptide having delta-9 elongase activity and having an amino acid sequence as set forth in SEQ ID NO:22, wherein SEQ ID NO:22 differs from SEQ ID NO:10 by at least one amino acid mutation, said mutation(s) selected from the group consisting of:
 - i) a L35F mutation;
 - ii) a L35M mutation;
 - iii) a L35G mutation;
 - iv) a L35G mutation and at least one other mutation selected from the group consisting of: S9A, S9D, S9G, S9I, S9K, S9Q, Q12K, A21D, A21T, A21V, V32F, Y84C, Q107E, L108G, G127L, W132T, M143N, M143W, L161T, L161Y, W168G, I179M, I179R, C236N, Q244N, A254W and A254Y;
 - v) L35G, A21V, L108G and I179R mutations;
 - vi) L35G, W132T and I179 mutations;
 - vii) L35G, S9D, Y84C and I179R mutations;
 - viii) L35G, Y84C, I179R and Q244N mutations;
 - ix) L35G, A21V, W132T, I179R and Q244N mutations;
 - x) K58R and I257T mutations;
 - xi) a D98G mutation;
 - xii) L130M and V243A mutations; and,
 - i) any combination comprising at least two mutations, wherein the mutations are selected from the group consisting of: K58R, L35F, L35G, L35M, S9A, S9D, S9G, S9I, S9K, S9Q, Q12K, A21D, A21T, A21V, V32F, Y84C, D98G, Q107E, L108G, G127L, L130M, W132T, M143N, M143W, L161T, L161Y, W168G, I179M, I179R, C236N, V243A, Q244N, A254W, A254Y and I257T;
 - (b) a complement of the nucleotide sequence of part (a), wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

2. The isolated polynucleotide of Claim 1, wherein the nucleotide sequence is selected from the group consisting of SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:86, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:100, SEQ ID NO:103, SEQ ID NO:106 and SEQ ID NO:109.
3. A mutant polypeptide having delta-9 elongase activity encoded by the isolated polynucleotide of Claim 1.
4. The mutant polypeptide of Claim 3, wherein the protein sequence is selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:87, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107 and SEQ ID NO:110.
5. The mutant polypeptide of either of Claims 3 or 4, wherein the delta-9 elongase activity is at least about functionally equivalent to the delta-9 elongase activity of the polypeptide as set forth in SEQ ID NO:10.
6. The mutant polypeptide of Claim 5, wherein the percent substrate conversion of linoleic acid to eicosadienoic acid is at least 110% when compared to the percent substrate conversion of linoleic acid to eicosadienoic acid of the polypeptide as set forth in SEQ ID NO:10.
7. The mutant polypeptide of Claim 6, wherein the percent substrate conversion of linoleic acid to eicosadienoic acid is at least 120% when compared to the percent substrate conversion of linoleic acid to eicosadienoic acid of the polypeptide as set forth in SEQ ID NO:10.
8. A recombinant construct comprising the isolated polynucleotide of Claim 1, operably linked to at least one regulatory sequence.
9. A transformed cell comprising the isolated polynucleotide of Claim 1.

10. The transformed cell of Claim 9, wherein said cell is selected from the group consisting of: plants, bacteria, yeast, algae, euglenoids, stramenopiles, oomycetes and fungi.
11. The transformed cell of Claim 10, wherein the cell is an oleaginous yeast producing at least about 25% of its dry cell weight as oil.
12. The transformed cell of claim 11, wherein the oleaginous yeast further comprises at least one recombinant DNA construct comprising an isolated polynucleotide operably linked to at least one regulatory sequence, the recombinant DNA construct encoding a polypeptide selected from the group consisting of: delta-4 desaturase, delta-5 desaturase, delta-8 desaturase, delta-6 desaturase, delta-9 desaturase, delta-12 desaturase, delta-15 desaturase, delta-17 desaturase, C_{14/16} elongase, C_{16/18} elongase, C_{18/20} elongase and C_{20/22} elongase.
13. The transformed cell of claim 12, wherein oil produced by the oleaginous yeast comprises a long-chain polyunsaturated fatty acid selected from the group consisting of: arachidonic acid, eicosadienoic acid, eicosapentaenoic acid, eicosatetraenoic acid, eicosatrienoic acid, dihomo- γ -linolenic acid, docosatetraenoic acid, docosapentaenoic acid and docosahexaenoic acid.
14. The transformed cell of Claim 10 or Claim 11, wherein the oleaginous yeast is selected from the group consisting of: *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodospiridium*, *Cryptococcus*, *Trichosporon* and *Lipomyces*.
15. The transformed cell of Claim 14, wherein the cell is a *Yarrowia lipolytica*.
16. A method for producing a polyunsaturated fatty acid selected from the group consisting of: arachidonic acid, eicosadienoic acid, eicosapentaenoic acid, eicosatetraenoic acid, eicosatrienoic acid, dihomo- γ -linolenic acid,

docosatetraenoic acid, docosapentaenoic acid and docosahexaenoic acid, the method comprising:

- a) providing an oleaginous yeast comprising:
 - i) a recombinant construct, operably linked to at least one regulatory sequence, wherein said recombinant construct comprises an isolated polynucleotide encoding a mutant polypeptide having delta-9 elongase activity and having an amino acid sequence as set forth in SEQ ID NO:22, wherein SEQ ID NO:22 differs from SEQ ID NO:10 by at least one amino acid mutation, said mutation(s) selected from the group consisting of:
 - (a) a L35F mutation;
 - (b) a L35M mutation;
 - (c) a L35G mutation;
 - (d) a L35G mutation and at least one other mutation selected from the group consisting of: S9A, S9D, S9G, S9I, S9K, S9Q, Q12K, A21D, A21T, A21V, V32F, Y84C, Q107E, L108G, G127L, W132T, M143N, M143W, L161T, L161Y, W168G, I179M, I179R, C236N, Q244N, A254W and A254Y;
 - (e) L35G, A21V, L108G and I179R mutations;
 - (f) L35G, W132T and I179 mutations;
 - (g) L35G, S9D, Y84C and I179R mutations;
 - (h) L35G, Y84C, I179R and Q244N mutations;
 - (i) L35G, A21V, W132T, I179R and Q244N mutations;
 - (j) K58R and I257T mutations;
 - (k) a D98G mutation;
 - (l) L130M and V243A mutations; and,
 - (m) any combination comprising at least two mutations, wherein the mutations are selected from the group consisting of: K58R, L35F, L35G, L35M, S9A, S9D, S9G, S9I, S9K, S9Q, Q12K, A21D, A21T, A21V, V32F, Y84C, D98G, Q107E, L108G, G127L, L130M, W132T, M143N, M143W, L161T, L161Y, W168G, I179M, I179R, C236N, V243A, Q244N, A254W, A254Y and I257T; and,

- ii) a source of substrate fatty acid selected from the group consisting of linoleic acid and alpha-linolenic acid;
 - b) growing the yeast of step (a) under conditions wherein the recombinant construct encoding the mutant polypeptide having delta-9 elongase activity is expressed and the substrate fatty acid is converted to product fatty acid, wherein linoleic acid is converted to eicosadienoic acid and alpha-linolenic acid is converted to eicosatrienoic acid; and,
 - c) optionally recovering the product fatty acid of step (b).
17. Microbial oil obtained from the oleaginous yeast of claim 11.
18. A recombinant microbial host cell producing an oil comprising at least 22.5 weight percent of eicosapentaenoic acid measured as a weight percent of dry cell weight, said recombinant microbial host cell comprising at least one mutant delta-9 elongase polypeptide, wherein said mutant delta-9 elongase polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:59.

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E389D9e (SEQ ID NO:4)	1	MAAVIEVANEFAVTAETAEIKPKVDYQRIEEDDIYSCHEHNSIAFVIINFTELSELSDSGKK-	60
Ead9e (SEQ ID NO:12)	(1)	-----MEAAKELVSIQVESEKVDYAQIMEDASSCEVNLVSAPVAIRFMRPLDLKRQA-	
Egd9e (SEQ ID NO:8)	(1)	-----MEVVNEIVSICQVEKVDYAQIMEDASHCHETVLSIAFVIINFTELSELCKQS-	
Igd9e (SEQ ID NO:2)	(1)	-----MAKANDAGERMAAVTDPEHIGTSTYILLMPILRNISGIVDEKK	
Consensus	(1)	MEVANEIVSI QE LKVDYARIMADASSCEVNLVSIAFVIINFTELSELCKGQK	
E389D9e (SEQ ID NO:4)	61	* * * * *	* 120
Ead9e (SEQ ID NO:12)	(60)	-ILRVLEKWINLFMSVLELVFELCMGPIHIVTG-----LYSNECDRAFDN	
Egd9e (SEQ ID NO:8)	(56)	-TLKKLETAIFLMSIVSESGFELAMAYELSVTG-----ILSGDCETAENN	
Igd9e (SEQ ID NO:2)	(56)	-RMKFVTNAILLMSIVSESGFELSMAYMYTIG-----VMSDNCEKAEDN	
Consensus	(45)	GAYETSMIWINVLLIALHILFIVTATPLGWDYGTGAWLRQGTGETFPQLEQCFSPVWDS	
	(61)	LK LETWYNLLMSIVSESGFELAMAYELSVTIG ILSNICEKAEDN	
E389D9e (SEQ ID NO:4)	121	** * * * *	180
Ead9e (SEQ ID NO:12)	(104)	SLEFATKVEVAVKKELEHDSFYELIMAKELSELQEFHEKAPADMMWEFYQVSCSSIMIF	
Egd9e (SEQ ID NO:8)	(100)	DVERITIQLEFLDKRYEHLDSFYELIMDKPLSELQEFHEKAPADMMWEFYKYRNEGWWIF	
Igd9e (SEQ ID NO:2)	(100)	NVPRITIQLEFLDKRYEHLDSFYELIMDKPLSELQEFHEKAPADMMWEFYNYRNEGWWIF	
Consensus	(105)	KLEPTWAKAEVAVKRYEHLDSFYELIMDKRVSELQEFHEKAPADMMWEFYRLHNEGWWIF	
	(121)	LEPRITQLEVAVKRYEHLDSFYELIMDKPLSELQEFHEKAPADMMWEFYQYRNEGWWIF	
E389D9e (SEQ ID NO:4)	181	* * * * *	240
Ead9e (SEQ ID NO:12)	(164)	VFLMAEFTHVWNGYVWIRLANKFNEPMPKLLITAMQITQMVGHYLVMAKNDIPCYRKDEPM	
Egd9e (SEQ ID NO:8)	(160)	VLLMAEFTHVWNGYVWIRLKLKNEPMPKLLITSMQIHQENVGHYLVMAKNDIPCYRKDEPM	
Igd9e (SEQ ID NO:2)	(160)	VLLMAEFTHVWNGYVWIRLKLKNEPMPKLLITSMQIHQENVGHYLVMAKNDIPCYRKDEPM	
Consensus	(165)	MFPNGSIFTHVWNTYVSLTAAGYNKKAPELITAMQITQMVGHYLVMAKNDIPCYRKDEPM	
	(181)	VLLMAEFTHVWNGYVWIRLKLKNEPMPKLLITAMQITQMVGHYLVMAKNDIPCYRKDEPM	
E389D9e (SEQ ID NO:4)	241	** * * * *	* 280
Ead9e (SEQ ID NO:12)	(224)	RMLEMLINVMVGVGVLLLEHINFEVKSIVFPEKNTADKKVQ	
Egd9e (SEQ ID NO:8)	(220)	RMEMLINVMVGVGVLLLEHINFMVQYIYRKPRKNRGKKE-	
Igd9e (SEQ ID NO:2)	(220)	RMFGMLINVMVGVGVLLLEHINFMVQYIYVIRHKGAKKIQ-	
Consensus	(224)	KLEMLINVMVGVGVLLLEHCHFSYQDNLATKKSANAGKQL	
	(241)	RMEMLINVMVGVGVLLLEHINFEVQYIYI KKKKAAAKKQ	

FIG. 1

2/15

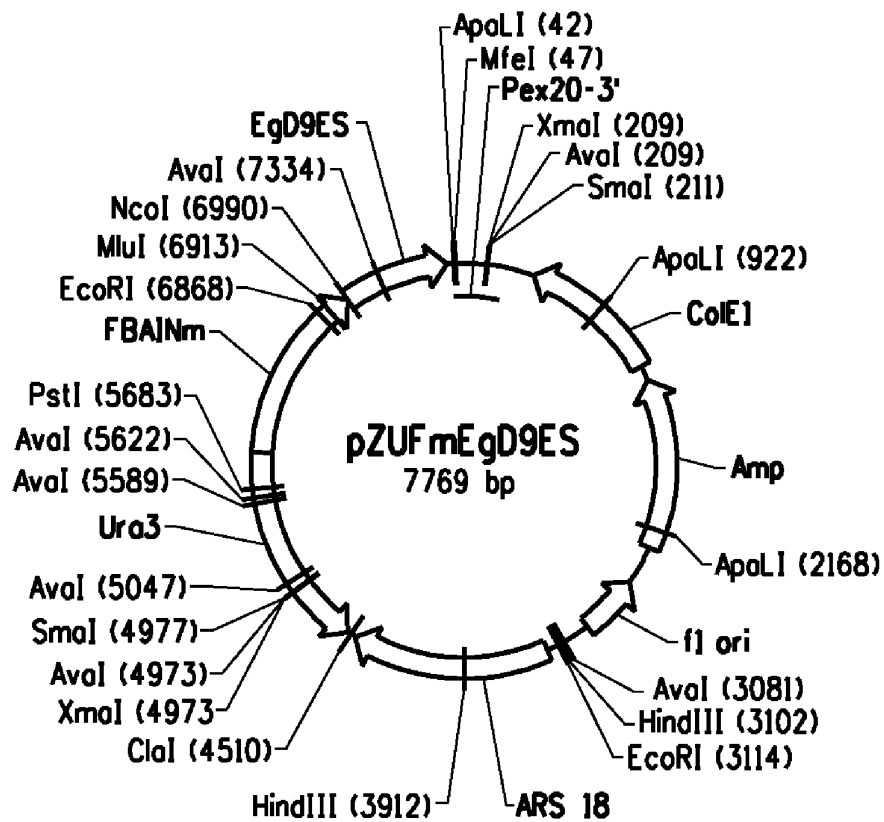


FIG. 2

3415

[illegible]

34
35
36
37
38

415

[illegible]

3
2
1
0
-1
-2

9/15

2010-01-01

Cl_elo = SEQ ID NO:43
Om_elo = SEQ ID NO:44
Mp_elo1 = SEQ ID NO:45
Pp_elo1 = SEQ ID NO:46
Mp_d5e = SEQ ID NO:47
Ot_elo1 = SEQ ID NO:48
Pav_elo2 = SEQ ID NO:49
Ps_elo2 = SEQ ID NO:50
Ot_elo2 = SEQ ID NO:51
Ea_d9e = SEQ ID NO:12
Eg_d9e = SEQ ID NO:8
E398_d9e = SEQ ID NO:4
lg_d9e = SEQ ID NO:2
Tp_elo2 = SEQ ID NO:52
Tp_elo1 = SEQ ID NO:53
Ma_d6e = SEQ ID NO:54
Th_elo2 = SEQ ID NO:55

Ci_elc	289
Om_elc	295
Mp_elcl	290
Pp_elcl	290
Mp_d5e	348
Ot_elcl	292
Pav_elcl2	277
Ps_elcl2	302
Ot_elcl2	300
Ea_d5e	258
Eg_elcl	258
E389_d5e	263
Ig_d5e	263
Tp_elcl2	SKAVERKSTRTIGAM 358
Tp_elcl	272
Ma_d5e	317
Th_elcl2	271
Trace_1	382
Trace_2	310
Trace_3	280
Trace_4	schaqqghsqashhf 370

30

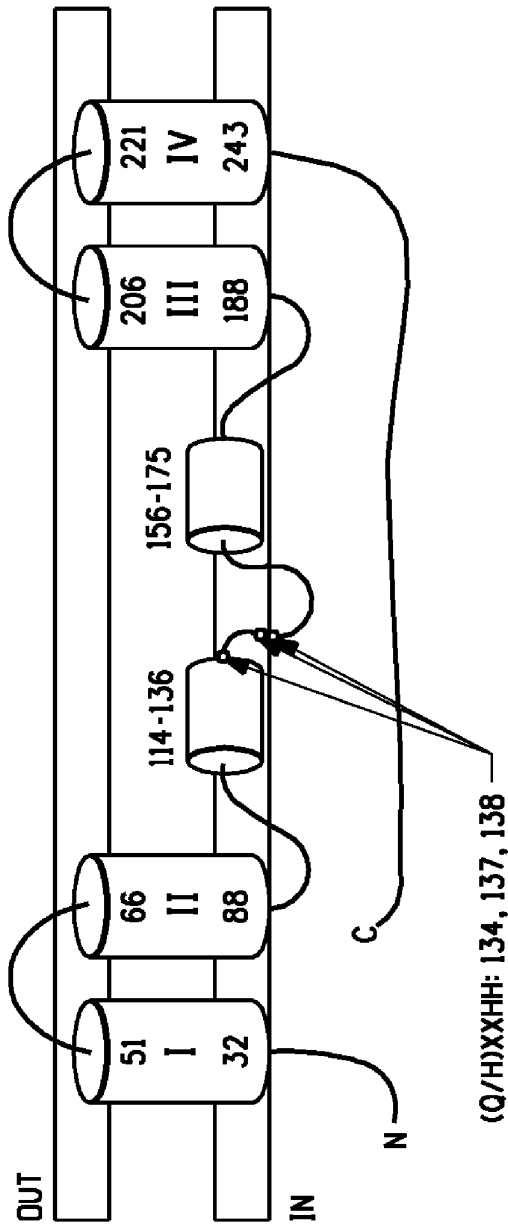


FIG. 4A

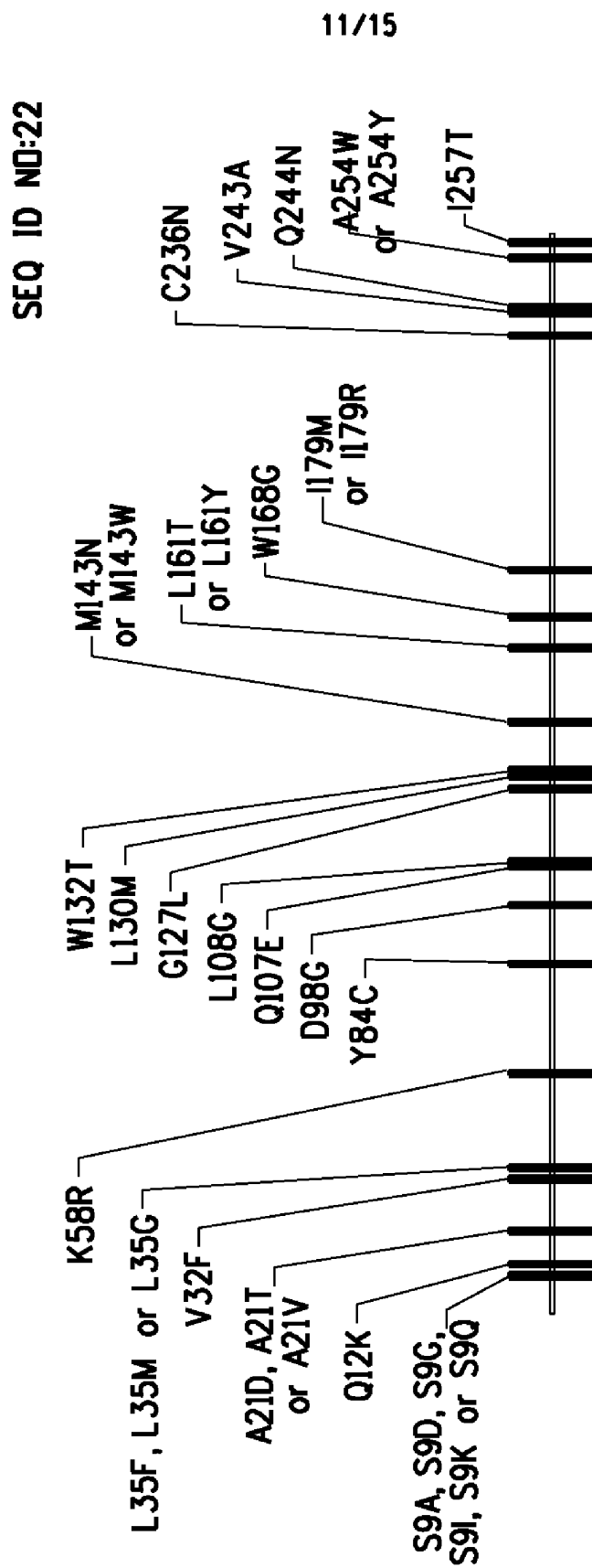
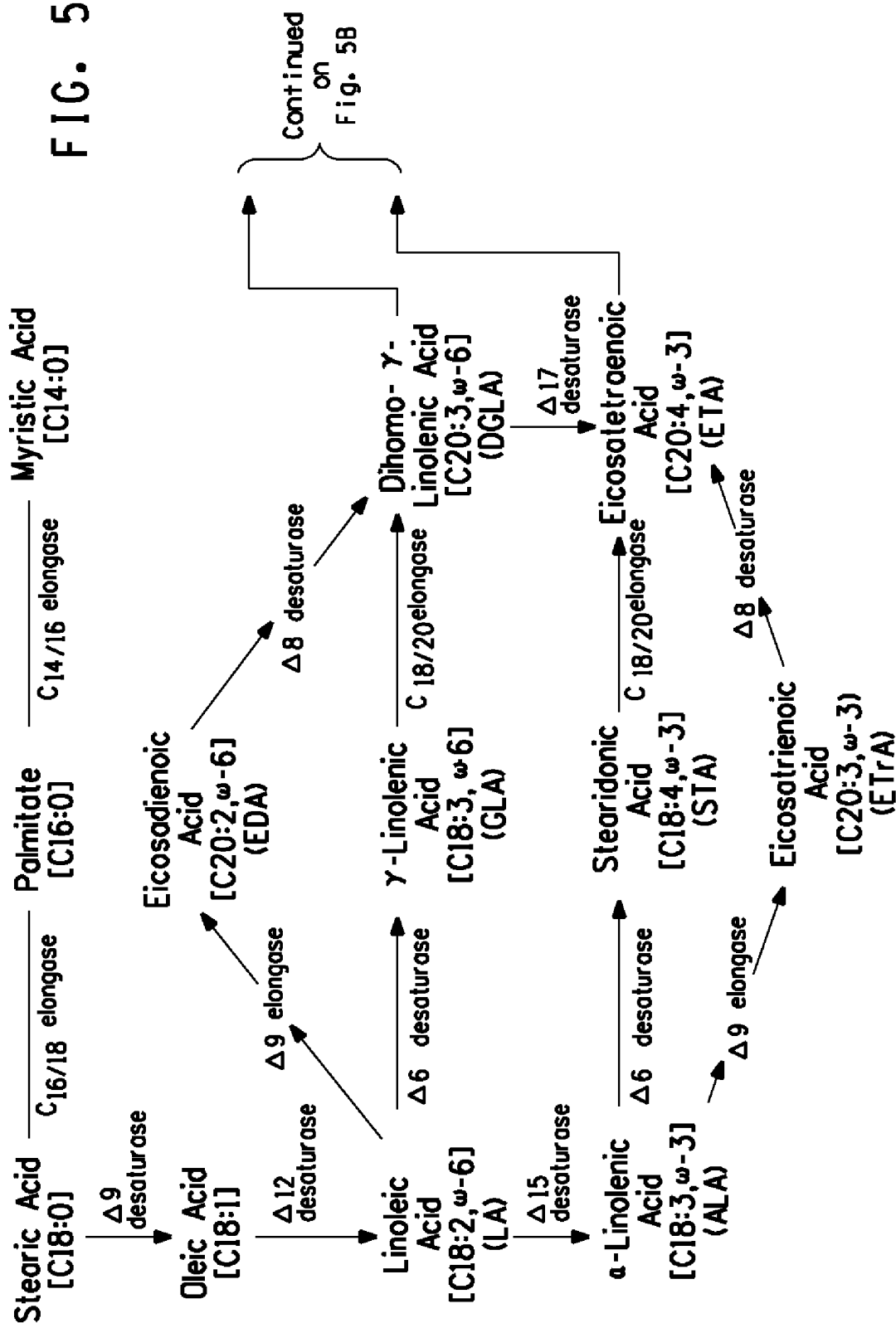


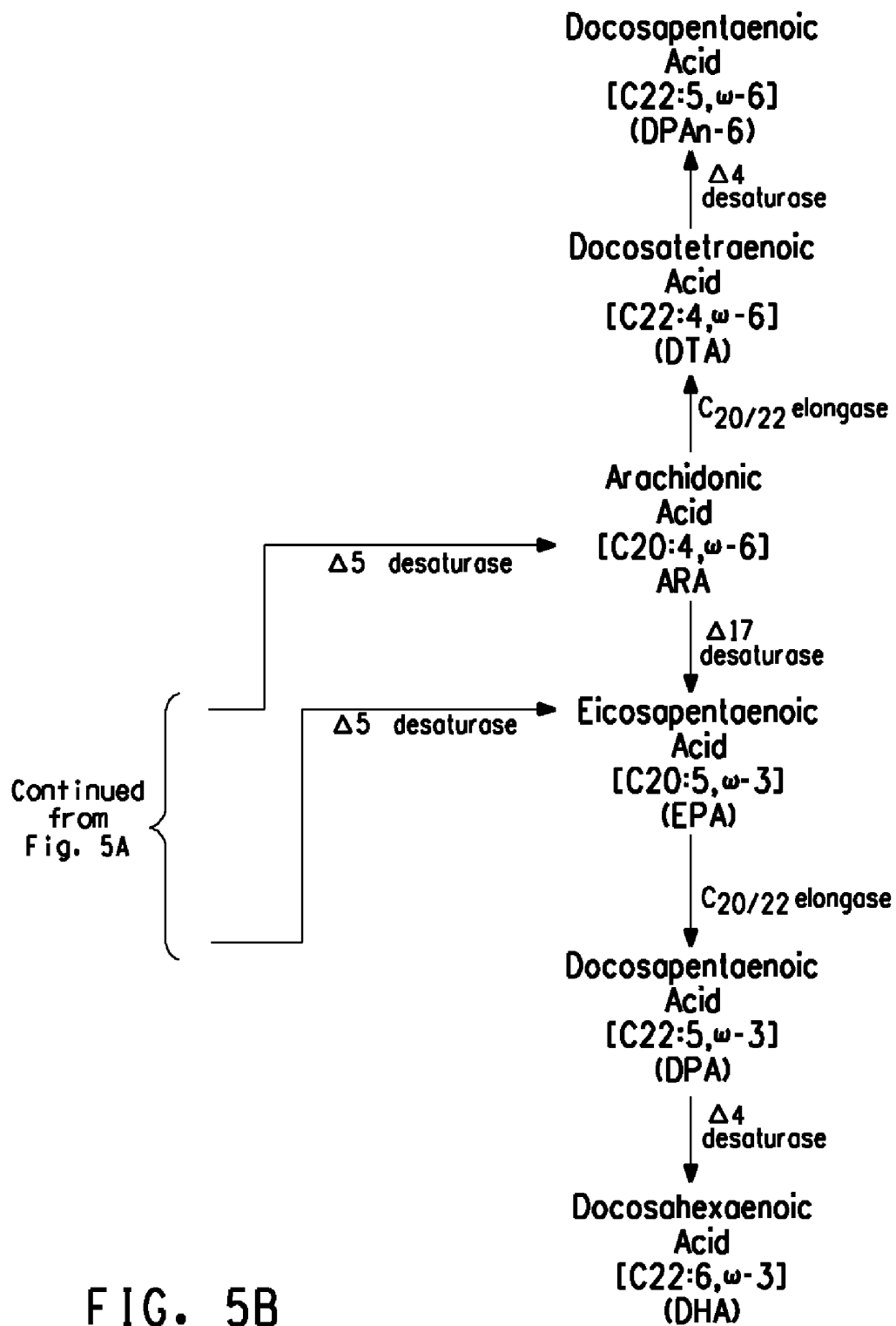
FIG. 4B

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FIG. 5A



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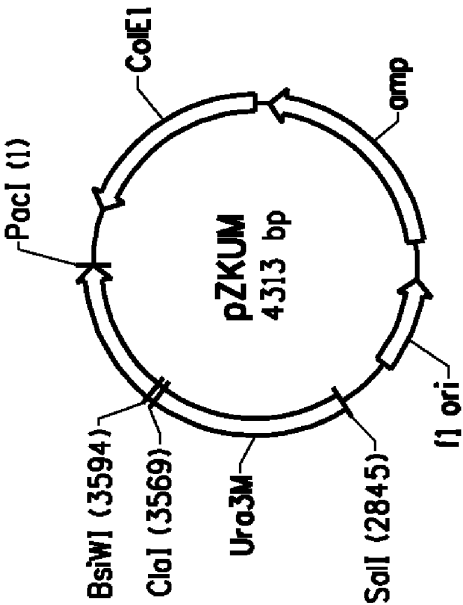


FIG. 7A

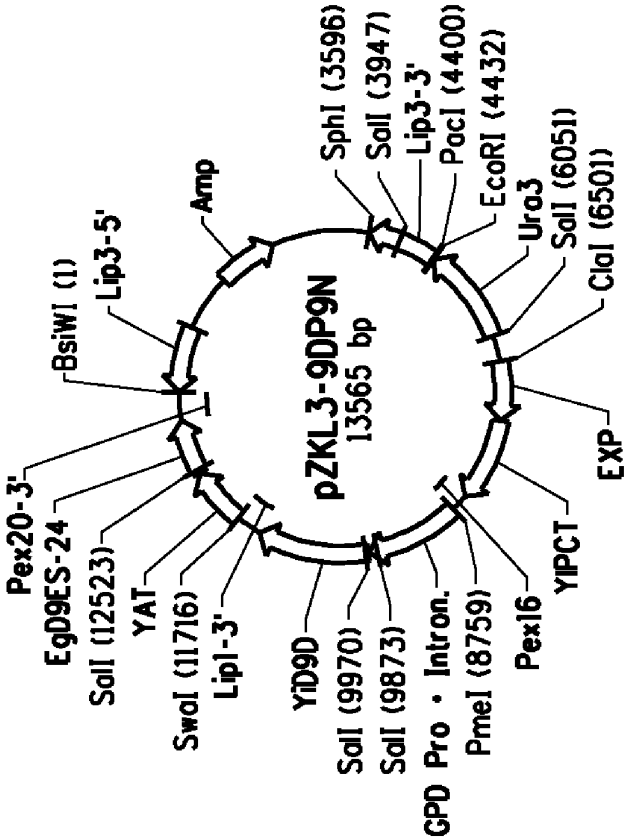


FIG. 7B

20110826_CL4783WOPCT_SequenceListing_ST25
SEQUENCE LISTING

<110> E.I. duPont de Nemours and Company, Inc.
Li, Yougen
Bostick, Michael
Zhu, Quinn

<120> MUTANT DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED

<130> CL4783

<150> US 61/377,248
<151> 2010-08-26

<160> 111

<170> PatentIn version 3.5

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<212> DNA
<213> Isochrysis galbana (GenBank Accession No. AF390174)

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<222> (2)..(793)
<223> delta-9 elongase (IgD9e)

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gac ccg gaa atc ctc att ggc acc ttc tcg tac ttg cta ctc aaa ccg 97
Asp Pro Glu Ile Leu Ile Gly Thr Phe Ser Tyr Leu Leu Leu Lys Pro
20 25 30
ctg ctc cgc aat tcc ggg ctg gtg gat gag aag aag ggc gca tac agg 145
Leu Leu Arg Asn Ser Gly Leu Val Asp Glu Lys Lys Gly Ala Tyr Arg
35 40 45
acg tcc atg atc tgg tac aac gtt ctg ctg gcg ctc ttc tct gcg ctg 193
Thr Ser Met Ile Trp Tyr Asn Val Leu Leu Ala Leu Phe Ser Ala Leu
50 55 60
agc ttc tac gtg acg gcg acc gcc ctc ggc tgg gac tat ggt acg ggc 241
Ser Phe Tyr Val Thr Ala Thr Ala Leu Gly Trp Asp Tyr Gly Thr Gly
65 70 75 80
gcg tgg ctg cgc agg caa acc ggc gac aca ccg cag ccg ctc ttc cag 289
Ala Trp Leu Arg Arg Gln Thr Gly Asp Thr Pro Gln Pro Leu Phe Gln
85 90 95
tgc ccg tcc ccg gtt tgg gac tcg aag ctc ttc aca tgg acc gcc aag 337
Cys Pro Ser Pro Val Trp Asp Ser Lys Leu Phe Thr Trp Thr Ala Lys
100 105 110
gca ttc tat tac tcc aag tac gtg gag tac ctc gac acg gcc tgg ctg 385
Ala Phe Tyr Tyr Ser Lys Tyr Val Glu Tyr Leu Asp Thr Ala Trp Leu
115 120 125
gtg ctc aag ggc aag agg gtc tcc ttt ctc cag gcc ttc cac cac ttt 433
Val Leu Lys Gly Lys Arg Val Ser Phe Leu Gln Ala Phe His His Phe
130 135 140
ggc gcg ccg tgg gat gtg tac ctc ggc att cgg ctg cac aac gag ggc 481
Gly Ala Pro Trp Asp Val Tyr Leu Gly Ile Arg Leu His Asn Glu Gly

20110826_CL4783WOPCT_SequenceListing_ST25

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gta tgg atc ttc atg ttt ttc aac tcg ttc att cac acc atc atg tac	Val Trp Ile Phe Met Phe Phe Asn Ser Phe Ile His Thr Ile Met Tyr	529		
	165	170	175	
acc tac tac ggc ctc acc gcc gcc ggg tat aag ttc aag gcc aag ccg	Thr Tyr Tyr Gly Leu Thr Ala Ala Gly Tyr Lys Phe Lys Ala Lys Pro	577		
	180	185	190	
ctc atc acc gcg atg cag atc tgc cag ttc gtg ggc ggc ttc ctg ttg	Leu Ile Thr Ala Met Gln Ile Cys Gln Phe Val Gly Gly Phe Leu Leu	625		
	195	200	205	
gtc tgg gac tac atc aac gtc ccc tgc ttc aac tcg gac aaa ggg aag	Val Trp Asp Tyr Ile Asn Val Pro Cys Phe Asn Ser Asp Lys Gly Lys	673		
	210	215	220	
ttg ttc agc tgg gct ttc aac tat gca tac gtc ggc tcg gtc ttc ttg	Leu Phe Ser Trp Ala Phe Asn Tyr Ala Tyr Val Gly Ser Val Phe Leu	721		
	225	230	235	
ctc ttc tgc cac ttt ttc tac cag gac aac ttg gca acg aag aaa tcg	Leu Phe Cys His Phe Phe Tyr Gln Asp Asn Leu Ala Thr Lys Lys Ser	769		
	245	250	255	
gcc aag gcg ggc aag cag ctc tag gcctcgagcc ggctcgcggg ttcaaggagg	Ala Lys Ala Gly Lys Gln Leu	823		
	260			
gcgacacggg ggtgggacgt ttgcatggag atggattgtg gatgtcctta cgccttactc		883		
atcaatgtcc tcccatctct cccctctaga ctttctacta gccatctaga agggcagctc		943		
agagacggat accgttcccc ctccccttcc ttttcgtctt tgctttgcca ttgtttgttt		1003		
gtctctattt tttaaactat tgacgctaac gcgttacgct cgcaaaaaaa aaaaaaaaaa		1063		
a		1064		

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Leu Leu Arg Asn Ser Gly Leu Val Asp Glu Lys Lys Gly Ala Tyr Arg	35	40	45	
Thr Ser Met Ile Trp Tyr Asn Val Leu Leu Ala Leu Phe Ser Ala Leu	50	55	60	
Ser Phe Tyr Val Thr Ala Thr Ala Leu Gly Trp Asp Tyr Gly Thr Gly	65	70	75	80

20110826_CL4783WOPCT_SequenceListing_ST25

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85 90 95

Cys Pro Ser Pro Val Trp Asp Ser Lys Leu Phe Thr Trp Thr Ala Lys
100 105 110

Ala Phe Tyr Tyr Ser Lys Tyr Val Glu Tyr Leu Asp Thr Ala Trp Leu
115 120 125

Val Leu Lys Gly Lys Arg Val Ser Phe Leu Gln Ala Phe His His Phe
130 135 140

Gly Ala Pro Trp Asp Val Tyr Leu Gly Ile Arg Leu His Asn Glu Gly
145 150 155 160

Val Trp Ile Phe Met Phe Phe Asn Ser Phe Ile His Thr Ile Met Tyr
165 170 175

Thr Tyr Tyr Gly Leu Thr Ala Ala Gly Tyr Lys Phe Lys Ala Lys Pro
180 185 190

Leu Ile Thr Ala Met Gln Ile Cys Gln Phe Val Gly Gly Phe Leu Leu
195 200 205

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

Leu Phe Ser Trp Ala Phe Asn Tyr Ala Tyr Val Gly Ser Val Phe Leu
225 230 235 240

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Ala Lys Ala Gly Lys Gln Leu
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<222> (1)..(792)
<223> delta-9 elongase

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ACIDS
<310> WO 2007/061742
<311> 2006-11-16
<312> 2007-05-31
<313> (1)..(792)

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20110826_CL4783WOPCT_SequenceListing_ST25

<302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
 <310> US 7,645,604
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 <312> 2010-01-12
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gaa acg ctc ccc aaa gtt gac tat caa cga cta tgg cga gac att tac	96
Glu Thr Leu Pro Lys Val Asp Tyr Gln Arg Leu Trp Arg Asp Ile Tyr	
20 25 30	
agt tgt gag cta ctg tat ttc tcc att gcc ttc gtg atc ttg aag ttt	144
Ser Cys Glu Leu Leu Tyr Phe Ser Ile Ala Phe Val Ile Leu Lys Phe	
35 40 45	
acg ttg ggc gag ttg agc gac agc gga aaa aag att ttg aga gtg ttg	192
Thr Leu Gly Glu Leu Ser Asp Ser Gly Lys Lys Ile Leu Arg Val Leu	
50 55 60	
ttc aag tgg tac aat ctc ttc atg tcc gtg ttc tcc ttg gtg tct ttc	240
Phe Lys Trp Tyr Asn Leu Phe Met Ser Val Phe Ser Leu Val Ser Phe	
65 70 75 80	
ctt tgc atg ggc tat gcc att tat acc gtg ggc cta tac tct aac gaa	288
Leu Cys Met Gly Tyr Ala Ile Tyr Thr Val Gly Leu Tyr Ser Asn Glu	
85 90 95	
tgc gac agg gct ttc gac aac tcg ttg ttc cgc ttt gca aca aag gtg	336
Cys Asp Arg Ala Phe Asp Asn Ser Leu Phe Arg Phe Ala Thr Lys Val	
100 105 110	
ttc tac tac agt aag ttt ttg gag tac atc gac tct ttt tat ctt ccg	384
Phe Tyr Tyr Ser Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro	
115 120 125	
ctc atg gcc aag ccg ctg tct ttc ctg caa ttc ttc cat cac ttg gga	432
Leu Met Ala Lys Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly	
130 135 140	
gcc ccc atg gac atg tgg ctc ttt gtc caa tat tct ggg gaa tct att	480
Ala Pro Met Asp Met Trp Leu Phe Val Gln Tyr Ser Gly Glu Ser Ile	
145 150 155 160	
tgg atc ttt gtg ttt ttg aat ggg ttc att cac ttt gtt atg tac ggg	528
Trp Ile Phe Val Phe Leu Asn Gly Phe Ile His Phe Val Met Tyr Gly	
165 170 175	
tac tac tgg act cgg ctg atg aag ttc aat ttc cca atg ccc aag cag	576
Tyr Tyr Trp Thr Arg Leu Met Lys Phe Asn Phe Pro Met Pro Lys Gln	
180 185 190	
ttg att acc gcg atg cag atc acg cag ttc aac gtt ggt ttc tac ctc	624
Leu Ile Thr Ala Met Gln Ile Thr Gln Phe Asn Val Gly Phe Tyr Leu	
195 200 205	
gtg tgg tgg tac aaa gat att ccc tgc tac cga aag gat ccc atg cga	672
Val Trp Trp Tyr Lys Asp Ile Pro Cys Tyr Arg Lys Asp Pro Met Arg	
210 215 220	
atg ttg gcc tgg atc ttc aat tac tgg tat gtt ggg act gtc ttg ctg	720
Met Leu Ala Trp Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu	
225 230 235 240	

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ctg ttc att aat ttc ttc gtc aaa tcc tat gtg ttc cca aag ccg aag 768
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 245 250 255

act gca gat aaa aag gtc caa tag 792
 Thr Ala Asp Lys Lys Val Gln
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 20 25 30

Ser Cys Glu Leu Leu Tyr Phe Ser Ile Ala Phe Val Ile Leu Lys Phe
 35 40 45

Thr Leu Gly Glu Leu Ser Asp Ser Gly Lys Lys Ile Leu Arg Val Leu
 50 55 60

Phe Lys Trp Tyr Asn Leu Phe Met Ser Val Phe Ser Leu Val Ser Phe
 65 70 75 80

Leu Cys Met Gly Tyr Ala Ile Tyr Thr Val Gly Leu Tyr Ser Asn Glu
 85 90 95

Cys Asp Arg Ala Phe Asp Asn Ser Leu Phe Arg Phe Ala Thr Lys Val
 100 105 110

Phe Tyr Tyr Ser Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro
 115 120 125

Leu Met Ala Lys Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly
 130 135 140

Ala Pro Met Asp Met Trp Leu Phe Val Gln Tyr Ser Gly Glu Ser Ile
 145 150 155 160

Trp Ile Phe Val Phe Leu Asn Gly Phe Ile His Phe Val Met Tyr Gly
 165 170 175

Tyr Tyr Trp Thr Arg Leu Met Lys Phe Asn Phe Pro Met Pro Lys Gln
 180 185 190

Leu Ile Thr Ala Met Gln Ile Thr Gln Phe Asn Val Gly Phe Tyr Leu
 195 200 205

20110826_CL4783WOPCT_SequenceListing_ST25

Val Trp Trp Tyr Lys Asp Ile Pro Cys Tyr Arg Lys Asp Pro Met Arg
210 215 220

Met Leu Ala Trp Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu
225 230 235 240

Leu Phe Ile Asn Phe Phe Val Lys Ser Tyr Val Phe Pro Lys Pro Lys
245 250 255

Thr Ala Asp Lys Lys Val Gln
260

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<302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
<310> WO 2007/061742
<311> 2006-11-16
<312> 2007-05-31
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<310> US 7,645,604
<311> 2006-11-16
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Glu Thr Leu Pro Lys Val Asp Tyr Gln Arg Leu Trp Arg Asp Ile Tyr
20 25 30

tcc tgc gag ctc ctg tac ttc tcc att gct ttc gtc atc ctc aag ttt 144
Ser Cys Glu Leu Leu Tyr Phe Ser Ile Ala Phe Val Ile Leu Lys Phe
35 40 45

acc ctt ggc gag ctc tcg gat tct ggc aaa aag att ctg cga gtg ctg 192
Thr Leu Gly Glu Leu Ser Asp Ser Gly Lys Lys Ile Leu Arg Val Leu
50 55 60

ttc aag tgg tac aac ctc ttc atg tcc gtc ttt tcg ctg gtg tcc ttc 240
Phe Lys Trp Tyr Asn Leu Phe Met Ser Val Phe Ser Leu Val Ser Phe
65 70 75 80

ctc tgt atg ggt tac gcc atc tac acc gtt gga ctg tac tcc aac gaa 288
Leu Cys Met Gly Tyr Ala Ile Tyr Thr Val Gly Leu Tyr Ser Asn Glu
85 90 95

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ttc tac tat tcc aag ttt ctg gag tac atc gac tct ttc tac ctt ccc Phe Tyr Tyr Ser Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro 115 120 125	384
ctc atg gcc aag cct ctg tcc ttt ctg cag ttc ttt cat cac ttg gga Leu Met Ala Lys Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly 130 135 140	432
gct cct atg gac atg tgg ctc ttc gtg cag tac tct ggc gaa tcc att Ala Pro Met Asp Met Trp Leu Phe Val Gln Tyr Ser Gly Glu Ser Ile 145 150 155 160	480
tgg atc ttt gtg ttc ctg aac gga ttc att cac ttt gtc atg tac ggc Trp Ile Phe Val Phe Leu Asn Gly Phe Ile His Phe Val Met Tyr Gly 165 170 175	528
tac tat tgg aca cgg ctg atg aag ttc aac ttt ccc atg ccc aag cag Tyr Tyr Trp Thr Arg Leu Met Lys Phe Asn Phe Pro Met Pro Lys Gln 180 185 190	576
ctc att acc gca atg cag atc acc cag ttc aac gtt ggc ttc tac ctc Leu Ile Thr Ala Met Gln Ile Thr Gln Phe Asn Val Gly Phe Tyr Leu 195 200 205	624
gtg tgg tgg tac aag gac att ccc tgt tac cga aag gat ccc atg cga Val Trp Trp Tyr Lys Asp Ile Pro Cys Tyr Arg Lys Asp Pro Met Arg 210 215 220	672
atg ctg gcc tgg atc ttc aac tac ttg tac gtc ggt acc gtt ctt ctg Met Leu Ala Trp Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu 225 230 235 240	720
ctc ttc atc aac ttc ttt gtc aag tcc tac gtg ttt ccc aag cct aag Leu Phe Ile Asn Phe Phe Val Lys Ser Tyr Val Phe Pro Lys Pro Lys 245 250 255	768
act gcc gac aaa aag gtc cag tag Thr Ala Asp Lys Lys Val Gln 260	792

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 <211> 263
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Ser Cys Glu Leu Leu Tyr Phe Ser Ile Ala Phe Val Ile Leu Lys Phe 35 40 45
Thr Leu Gly Glu Leu Ser Asp Ser Gly Lys Lys Ile Leu Arg Val Leu 50 55 60

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Phe Lys Trp Tyr Asn Leu Phe Met Ser Val Phe Ser Leu Val Ser Phe
65 70 75 80

Leu Cys Met Gly Tyr Ala Ile Tyr Thr Val Gly Leu Tyr Ser Asn Glu
85 90 95

Cys Asp Arg Ala Phe Asp Asn Ser Leu Phe Arg Phe Ala Thr Lys Val
100 105 110

Phe Tyr Tyr Ser Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro
115 120 125

Leu Met Ala Lys Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly
130 135 140

Ala Pro Met Asp Met Trp Leu Phe Val Gln Tyr Ser Gly Glu Ser Ile
145 150 155 160

Trp Ile Phe Val Phe Leu Asn Gly Phe Ile His Phe Val Met Tyr Gly
165 170 175

Tyr Tyr Trp Thr Arg Leu Met Lys Phe Asn Phe Pro Met Pro Lys Gln
180 185 190

Leu Ile Thr Ala Met Gln Ile Thr Gln Phe Asn Val Gly Phe Tyr Leu
195 200 205

Val Trp Trp Tyr Lys Asp Ile Pro Cys Tyr Arg Lys Asp Pro Met Arg
210 215 220

Met Leu Ala Trp Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu
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ACIDS
<310> WO 2007/061742

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<311> 2006-11-16
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 Lys Val Asp Tyr Ala Gln Leu Trp Ser Asp Ala Ser His Cys Glu Val
 20 25 30
 ctt tac ttg tcc atc gca ttt gtc atc ttg aag ttc act ctt ggc ccc 144
 Leu Tyr Leu Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro
 35 40 45
 ctt ggt cca aaa ggt cag tct cgt atg aag ttt gtt ttc acc aat tac 192
 Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
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 aac ctt ctc atg tcc att tat tcg ttg gga tca ttc ctc tca atg gca 240
 Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
 65 70 75 80
 tat gcc atg tac acc atc ggt gtt atg tct gac aac tgc gag aag gct 288
 Tyr Ala Met Tyr Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
 85 90 95
 ttt gac aac aac gtc ttc agg atc acc acg cag ttg ttc tat ttg agc 336
 Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
 100 105 110
 aag ttc ctg gag tat att gac tcc ttc tat ttg cca ctg atg ggc aag 384
 Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
 115 120 125
 cct ctg acc tgg ttg caa ttc ttc cat cat ttg ggg gca ccg atg gat 432
 Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
 130 135 140
 atg tgg ctg ttc tat aat tac cga aat gaa gct gtt tgg att ttt gtg 480
 Met Trp Leu Phe Tyr Asn Tyr Arg Asn Glu Ala Val Trp Ile Phe Val
 145 150 155 160
 ctg ttg aat ggt ttc atc cac tgg atc atg tac ggt tat tat tgg acc 528
 Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
 165 170 175
 aga ttg atc aag ctg aag ttc ccc atg cca aaa tcc ctg att aca tca 576
 Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
 180 185 190
 atg cag atc att caa ttc aat gtt ggt ttc tac att gtc tgg aag tac 624
 Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
 195 200 205
 agg aac att ccc tgt tat cgc caa gat ggg atg agg atg ttt ggc tgg 672
 Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
 210 215 220

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ttc ttc aat tac ttt tat gtt ggc aca gtc ttg tgt ttg ttc ttg aat 720
Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
225 230 235 240

ttc tat gtg caa acg tat atc gtc agg aag cac aag gga gcc aaa aag 768
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245 250 255

att cag tga 777
Ile Gln

<210> 8
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<212> PRT
<213> Euglena gracilis
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Lys Val Asp Tyr Ala Gln Leu Trp Ser Asp Ala Ser His Cys Glu Val
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Leu Tyr Leu Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro
35 40 45

Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
50 55 60

Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
65 70 75 80

Tyr Ala Met Tyr Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
85 90 95

Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
100 105 110

Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
115 120 125

Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
130 135 140

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

Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
165 170 175

Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

20110826_CL4783WOPCT_SequenceListing_ST25

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
195 200 205

Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
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Phe Tyr Val Gln Thr Tyr Ile Val Arg Lys His Lys Gly Ala Lys Lys
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Ile Gln

<210> 9
<211> 777
<212> DNA
<213> Euglena gracilis

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<223> synthetic delta-9 elongase (codon-optimized for Yarrowia lipolytica)

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<302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
<310> WO 2007/061742
<311> 2006-11-16
<312> 2007-05-31
<313> (1)..(777)

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<302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
<310> US 7,645,604
<311> 2006-11-16
<312> 2010-01-12
<313> (1)..(777)

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aag gtc gac tat gct cag ctc tgg tct gat gcc tcg cac tgc gag gtg 96
Lys Val Asp Tyr Ala Gln Leu Trp Ser Asp Ala Ser His Cys Glu Val
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ctg tac ctc tcc atc gcc ttc gtc atc ctg aag ttc acc ctt ggt cct 144
Leu Tyr Leu Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro
35 40 45

ctc gga ccc aag ggt cag tct cga atg aag ttt gtg ttc acc aac tac 192
Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
50 55 60

aac ctg ctc atg tcc atc tac tcg ctg ggc tcc ttc ctc tct atg gcc 240
Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala

20110826_CL4783WOPCT_SequenceListing_ST25

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	85	90	95	
ttc gac aac aat gtc ttc cga atc acc act cag ctg ttc tac ctc agc	Phe Asp Asn Asn Val Phe Arg Ile Thr Gln Leu Phe Tyr Leu Ser	336		
	100	105	110	
aag ttc ctc gag tac att gac tcc ttc tat ctg ccc ctc atg ggc aag	Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys	384		
	115	120	125	
cct ctg acc tgg ttg cag ttc ttt cac cat ctc gga gct cct atg gac	Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp	432		
	130	135	140	
atg tgg ctg ttc tac aac tac cga aac gaa gcc gtt tgg atc ttt gtg	Met Trp Leu Phe Tyr Asn Tyr Arg Asn Glu Ala Val Trp Ile Phe Val	480		
	145	150	155	
ctg ctc aac ggc ttc att cac tgg atc atg tac ggc tac tat tgg acc	Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr	528		
	165	170	175	
cga ctg atc aag ctc aag ttc cct atg ccc aag tcc ctg att act tct	Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser	576		
	180	185	190	
atg cag atc att cag ttc aac gtt ggc ttc tac atc gtc tgg aag tac	Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr	624		
	195	200	205	
cgg aac att ccc tgc tac cga caa gat gga atg aga atg ttt ggc tgg	Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp	672		
	210	215	220	
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	225	230	235	
ttc tac gtg cag acc tac atc gtc cga aag cac aag gga gcc aaa aag	Phe Tyr Val Gln Thr Tyr Ile Val Arg Lys His Lys Gly Ala Lys Lys	768		
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 <213> Euglena gracilis

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Leu Tyr Leu Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro	35	40	45	

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 Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
 65 70 75 80
 Tyr Ala Met Tyr Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
 85 90 95
 Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
 100 105 110
 Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
 115 120 125
 Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
 130 135 140
 Met Trp Leu Phe Tyr Asn Tyr Arg Asn Glu Ala Val Trp Ile Phe Val
 145 150 155 160
 Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
 165 170 175
 Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
 180 185 190
 Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
 195 200 205
 Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
 210 215 220
 Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
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 Ile Gln

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<310> U.S. 7,794,701
<311> 2008-04-15
<312> 2010-09-14
<313> (1)..(774)

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<311> 2008-04-16
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ctt tac ctc tcg gtg gca ttc gtg gcg atc aag ttc atg ctg cgc cca      144
Leu Tyr Leu Ser Val Ala Phe Val Ala Ile Lys Phe Met Leu Arg Pro
                               35          40          45

ctg gac ctg aag cgc cag gcc acc ttg aag aag ctg ttc aca gca tac      192
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50                               55          60

aac ttc ctc atg tcg atc tat tcc ttt ggc tcc ttc ctg gcc atg gcc      240
Asn Phe Leu Met Ser Ile Tyr Ser Phe Gly Ser Phe Leu Ala Met Ala
65                               70          75          80

tat gcc cta tca gta act ggc atc ctc tcc ggc gac tgt gag acg gcg      288
Tyr Ala Leu Ser Val Thr Gly Ile Leu Ser Gly Asp Cys Glu Thr Ala
                               85          90          95

ttc aac aac gat gtg ttc agg atc aca act cag ctg ttc tac ctc agc      336
Phe Asn Asn Asp Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
                               100          105          110

aag ttc gta gag tac atc gac tcc ttc tac ctt ccc ctt atg gac aag      384
Lys Phe Val Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Asp Lys
                               115          120          125

cca ctg tcg ttc ctt cag ttc ttc cat cat ttg ggg gcc ccc att gac      432
Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly Ala Pro Ile Asp
130          135          140

atg tgg cta ttc tac aaa tac cgc aac gaa gga gtc tgg atc ttt gtc      480
Met Trp Leu Phe Tyr Lys Tyr Arg Asn Glu Gly Val Trp Ile Phe Val
145          150          155          160

ctg ttg aat ggg ttc att cac tgg atc atg tac ggt tac tat tgg acg      528
Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
                               165          170          175

cgg ctc atc aag ctg aac ttc ccc atg ccc aag aac ctg atc acc tcc      576
Arg Leu Ile Lys Leu Asn Phe Pro Met Pro Lys Asn Leu Ile Thr Ser
                               180          185          190

atg cag atc atc cag ttc aat gtc ggg ttc tac atc gtc tgg aag tac      624
Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr

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210	215	220	
atc ttc aac tac tgg tat gtc ggg acg gtc ttg ctg ctg ttc ctc aac	Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu Phe Leu Asn		720
225	230	235	240
ttt tac gtg cag acg tac atc cgg aag ccg agg aag aac cga ggg aag	Phe Tyr Val Gln Thr Tyr Ile Arg Lys Pro Arg Lys Asn Arg Gly Lys		768
	245	250	255
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Lys Glu			

<210> 12
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 <213> Euglena anabaena UTEX 373

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Leu Tyr Leu Ser Val Ala Phe Val Ala Ile Lys Phe Met Leu Arg Pro	35	40	45	
Leu Asp Leu Lys Arg Gln Ala Thr Leu Lys Lys Leu Phe Thr Ala Tyr	50	55	60	
Asn Phe Leu Met Ser Ile Tyr Ser Phe Gly Ser Phe Leu Ala Met Ala	65	70	75	80
Tyr Ala Leu Ser Val Thr Gly Ile Leu Ser Gly Asp Cys Glu Thr Ala	85	90	95	
Phe Asn Asn Asp Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser	100	105	110	
Lys Phe Val Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Asp Lys	115	120	125	
Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly Ala Pro Ile Asp	130	135	140	
Met Trp Leu Phe Tyr Lys Tyr Arg Asn Glu Gly Val Trp Ile Phe Val	145	150	155	160
Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr	165	170	175	

20110826_CL4783WOPCT_SequenceListing_ST25

Arg Leu Ile Lys Leu Asn Phe Pro Met Pro Lys Asn Leu Ile Thr Ser
180 185 190

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
195 200 205

Arg Asn Val Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Ala Trp
210 215 220

Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu Leu Phe Leu Asn
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Phe Tyr Val Gln Thr Tyr Ile Arg Lys Pro Arg Lys Asn Arg Gly Lys
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Lys Glu

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<213> Euglena anabaena UTEX 373

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<310> U.S. 7,794,701
<311> 2008-04-15
<312> 2010-09-14
<313> (1)..(774)

<300>
<302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
<310> WO 2008/128241
<311> 2008-04-16
<312> 2008-10-23
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aag gtg gac tac gcc cag ctc tgg cag gac gcc tcc tct tgc gag gtt 96
Lys Val Asp Tyr Ala Gln Leu Trp Gln Asp Ala Ser Ser Cys Glu Val
20 25 30

ctg tac ctc tcg gtc gct ttc gtg gcc atc aag ttc atg ctt cga cct 144
Leu Tyr Leu Ser Val Ala Phe Val Ala Ile Lys Phe Met Leu Arg Pro
35 40 45

ctg gac ctc aag cga caa gcc acc ctc aaa aag ctg ttc acc gca tac 192

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Asn	Phe	Leu	Met	Ser	Ile	Tyr	Ser	Phe	Gly	Ser	Phe	Leu	Ala	Met	Ala	
65					70				75						80	
tac	gct	ctc	tct	gtc	act	ggc	att	ctt	tcc	ggc	gat	tgt	gag	act	gcc	288
Tyr	Ala	Leu	Ser	Val	Thr	Gly	Ile	Leu	Ser	Gly	Asp	Cys	Glu	Thr	Ala	
				85					90					95		
ttc	aac	aat	gac	gtg	ttc	cga	atc	acc	act	cag	ctg	ttc	tac	ctc	agc	336
Phe	Asn	Asn	Asp	Val	Phe	Arg	Ile	Thr	Thr	Gln	Leu	Phe	Tyr	Leu	Ser	
			100					105					110			
aag	ttc	gtc	gag	tac	atc	gac	tcc	ttc	tac	ctt	ccc	ctc	atg	gac	aag	384
Lys	Phe	Val	Glu	Tyr	Ile	Asp	Ser	Phe	Tyr	Leu	Pro	Leu	Met	Asp	Lys	
		115					120					125				
ccc	ttg	tcg	ttt	ctg	cag	ttc	ttt	cac	cat	ctc	gga	gct	ccc	atc	gac	432
Pro	Leu	Ser	Phe	Leu	Gln	Phe	Phe	His	His	Leu	Gly	Ala	Pro	Ile	Asp	
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atg	tgg	ctg	ttc	tac	aag	tat	cga	aac	gaa	ggc	gtc	tgg	atc	ttt	gtt	480
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145					150					155					160	
ctg	ctc	aac	ggc	ttc	att	cac	tgg	atc	atg	tac	ggc	tac	tat	tgg	acg	528
Leu	Leu	Asn	Gly	Phe	Ile	His	Trp	Ile	Met	Tyr	Gly	Tyr	Tyr	Trp	Thr	
				165					170					175		
cga	ctc	atc	aag	ctg	aac	ttc	cct	atg	ccc	aag	aac	ctc	att	acc	tcc	576
Arg	Leu	Ile	Lys	Leu	Asn	Phe	Pro	Met	Pro	Lys	Asn	Leu	Ile	Thr	Ser	
			180					185					190			
atg	caa	att	atc	cag	ttc	aac	gtc	gga	ttc	tac	atc	gtc	tgg	aag	tac	624
Met	Gln	Ile	Ile	Gln	Phe	Asn	Val	Gly	Phe	Tyr	Ile	Val	Trp	Lys	Tyr	
		195					200					205				
cga	aac	gtg	ccc	tgc	tac	cgg	cag	gac	ggc	atg	cga	atg	ttt	gcc	tgg	672
Arg	Asn	Val	Pro	Cys	Tyr	Arg	Gln	Asp	Gly	Met	Arg	Met	Phe	Ala	Trp	
		210				215					220					
atc	ttc	aac	tac	tgg	tat	gtc	ggc	acg	gtg	ctg	ctt	ctg	ttc	ctc	aac	720
Ile	Phe	Asn	Tyr	Trp	Tyr	Val	Gly	Thr	Val	Leu	Leu	Leu	Phe	Leu	Asn	
225					230					235					240	
ttc	tac	gtc	cag	acc	tac	att	cgg	aag	cct	cga	aag	aac	cga	ggc	aaa	768
Phe	Tyr	Val	Gln	Thr	Tyr	Ile	Arg	Lys	Pro	Arg	Lys	Asn	Arg	Gly	Lys	
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aag	gag															774
Lys	Glu															

<210> 14
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Lys	Val	Asp	Tyr	Ala	Gln	Leu	Trp	Gln	Asp	Ala	Ser	Ser	Cys	Glu	Val
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20

25

30

Leu Tyr Leu Ser Val Ala Phe Val Ala Ile Lys Phe Met Leu Arg Pro
 35 40 45

Leu Asp Leu Lys Arg Gln Ala Thr Leu Lys Lys Leu Phe Thr Ala Tyr
 50 55 60

Asn Phe Leu Met Ser Ile Tyr Ser Phe Gly Ser Phe Leu Ala Met Ala
 65 70 75 80

Tyr Ala Leu Ser Val Thr Gly Ile Leu Ser Gly Asp Cys Glu Thr Ala
 85 90 95

Phe Asn Asn Asp Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
 100 105 110

Lys Phe Val Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Asp Lys
 115 120 125

Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly Ala Pro Ile Asp
 130 135 140

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

Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
 165 170 175

Arg Leu Ile Lys Leu Asn Phe Pro Met Pro Lys Asn Leu Ile Thr Ser
 180 185 190

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
 195 200 205

Arg Asn Val Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Ala Trp
 210 215 220

Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu Leu Phe Leu Asn
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Phe Tyr Val Gln Thr Tyr Ile Arg Lys Pro Arg Lys Asn Arg Gly Lys
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<300>
 <302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
 <310> US 7,645,604
 <311> 2006-11-16
 <312> 2010-01-12
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 <302> DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
 <310> U.S. 7,645,604
 <311> 2006-11-16
 <312> 2010-01-12
 <313> (1)..(15)

<400> 16

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 <223> X = Q or H

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<220>
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<223> Xaa = Cys [C] (synthetic codon-optimized) or Asn [N] (mutant)

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<223> Xaa = Ala [A] (synthetic codon-optimized) or (Trp [W] or Tyr
[Y]) (mutant)

<220>
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<223> Xaa = Ile [I] (synthetic codon-optimized) or Thr [T] (mutant)

<400> 22

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Leu Tyr Xaa Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro
35 40 45

Leu Gly Pro Lys Gly Gln Ser Arg Met Xaa Phe Val Phe Thr Asn Tyr
50 55 60

Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
65 70 75 80

Tyr Ala Met Xaa Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
85 90 95

Phe Xaa Asn Asn Val Phe Arg Ile Thr Thr Xaa Xaa Phe Tyr Leu Ser
100 105 110

Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Xaa Lys
115 120 125

Pro Xaa Thr Xaa Leu Gln Phe Phe His His Leu Gly Ala Pro Xaa Asp
130 135 140

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

Xaa Leu Asn Gly Phe Ile His Xaa Ile Met Tyr Gly Tyr Tyr Trp Thr
165 170 175

Arg Leu Xaa Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
195 200 205

Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Xaa Leu Phe Leu Asn
225 230 235 240

Phe Tyr Xaa Xaa Thr Tyr Ile Val Arg Lys His Lys Gly Xaa Lys Lys
245 250 255

Xaa Gln

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

Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
130 135 140

Met Trp Leu Phe Tyr Asn Tyr Arg Asn Glu Ala Val Trp Ile Phe Val
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Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
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Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
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Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
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Phe	Phe	Asn	Tyr	Phe	Tyr	Val	Gly	Thr	Val	Leu	Cys	Leu	Phe	Leu	Asn	
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Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
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Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
195 200 205

Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
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ctg tac ctc tcc atc gcc ttc gtc atc ctg aag ttc acc ctt ggt cct      144
Leu Tyr Leu Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro
35 40 45

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Leu Tyr Leu Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro 35 40 45
Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr 50 55 60
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Pro Met Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
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Arg Leu Ile Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
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Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
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20110826_CL4783WOPCT_SequenceListing_ST25

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 <213> Ciona intestinalis
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Pro His Met Gln Lys Phe Leu Trp Trp Lys Lys Tyr Ile Thr Met Leu
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Gln Leu Val Gln Phe Val Leu Ala Ile Tyr His Thr Ala Arg Ser Leu
210 215 220

Tyr Val Lys Cys Pro Ser Pro Val Trp Met His Trp Ala Leu Ile Leu
225 230 235 240

Tyr Ala Phe Ser Phe Ile Leu Leu Phe Ser Asn Phe Tyr Met His Ala
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Tyr Ile Lys Lys Ser Arg Lys Gly Lys Glu Asn Gly Ser Arg Gly Lys
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Gly Gly Val Ser Asn Gly Lys Glu Lys Leu His Ala Asn Gly Lys Thr
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<400> 44

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Pro Pro Thr Phe Ala Leu Thr Val Met Tyr Leu Leu Ile Val Trp Met
35 40 45

Gly Pro Lys Tyr Met Arg His Arg Gln Pro Val Ser Cys Arg Gly Leu
50 55 60

Leu Leu Val Tyr Asn Leu Gly Leu Thr Ile Leu Ser Phe Tyr Met Phe
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Tyr Glu Met Val Ser Ala Val Trp His Gly Asp Tyr Asn Phe Phe Cys
85 90 95

Gln Asp Thr His Ser Ala Gly Glu Thr Asp Thr Lys Ile Ile Asn Val
100 105 110

Leu Trp Trp Tyr Tyr Phe Ser Lys Leu Ile Glu Phe Met Asp Thr Phe
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Phe Phe Ile Leu Arg Lys Asn Asn His Gln Ile Thr Phe Leu His Ile
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Tyr His His Ala Ser Met Leu Asn Ile Trp Trp Phe Val Met Asn Trp
145 150 155 160

Val Pro Cys Gly His Ser Tyr Phe Gly Ala Ser Leu Asn Ser Phe Ile
165 170 175

His Val Leu Met Tyr Ser Tyr Tyr Gly Leu Ser Ala Val Pro Ala Leu
180 185 190

Arg Pro Tyr Leu Trp Trp Lys Lys Tyr Ile Thr Gln Val Gln Leu Ile
195 200 205

Gln Phe Phe Leu Thr Met Ser Gln Thr Ile Cys Ala Val Ile Trp Pro
210 215 220

Cys Asp Phe Pro Arg Gly Trp Leu Tyr Phe Gln Ile Phe Tyr Val Ile
225 230 235 240

Thr Leu Ile Ala Leu Phe Ser Asn Phe Tyr Ile Gln Thr Tyr Lys Lys
245 250 255

His Leu Val Ser Gln Lys Lys Glu Tyr His Gln Asn Gly Ser Val Ala
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Ser Leu Asn Gly His Val Asn Gly Val Thr Pro Thr Glu Thr Ile Thr
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His Arg Lys Val Arg Gly Asp
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<210> 45
<211> 290
<212> PRT
<213> Marchantia polymorpha

<400> 45

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

Gly Leu Ser Ser Tyr Leu Thr Phe Val Phe Leu Gly Leu Ile Val Ile
50 55 60

Lys Ser Leu Asp Leu Lys Pro Arg Ser Lys Glu Pro Ala Ile Leu Asn
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65

70

75

80

Leu Phe Val Ile Phe His Asn Phe Val Cys Phe Ala Leu Ser Leu Tyr
 85 90 95

Met Cys Val Gly Ile Val Arg Gln Ala Ile Leu Asn Arg Tyr Ser Leu
 100 105 110

Trp Gly Asn Ala Tyr Asn Pro Lys Glu Val Gln Met Gly His Leu Leu
 115 120 125

Tyr Ile Phe Tyr Met Ser Lys Tyr Ile Glu Phe Met Asp Thr Val Ile
 130 135 140

Met Ile Leu Lys Arg Asn Thr Arg Gln Ile Thr Val Leu His Val Tyr
 145 150 155 160

His His Ala Ser Ile Ser Phe Ile Trp Trp Ile Ile Ala Tyr His Ala
 165 170 175

Pro Gly Gly Glu Ala Tyr Phe Ser Ala Ala Leu Asn Ser Gly Val His
 180 185 190

Val Leu Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Leu Gly Lys Asn
 195 200 205

Glu Lys Ala Arg Arg Lys Tyr Leu Trp Trp Gly Lys Tyr Leu Thr Gln
 210 215 220

Leu Gln Met Phe Gln Phe Val Leu Asn Met Ile Gln Ala Tyr Tyr Asp
 225 230 235 240

Ile Lys Asn Asn Ser Pro Tyr Pro Gln Phe Leu Ile Gln Ile Leu Phe
 245 250 255

Tyr Tyr Met Ile Ser Leu Leu Ala Leu Phe Gly Asn Phe Tyr Val His
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Lys Tyr Val Ser Ala Pro Ala Lys Pro Ala Lys Ile Lys Ser Lys Lys
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Ala Glu
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<210> 46

<211> 290

<212> PRT

<213> Physcomitrella patens

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Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
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Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
20 25 30

Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
35 40 45

Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
50 55 60

Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65 70 75 80

Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
85 90 95

Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
100 105 110

Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
115 120 125

Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
130 135 140

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

Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
165 170 175

His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
180 185 190

Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
195 200 205

Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
210 215 220

Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225 230 235 240

Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
245 250 255

Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
260 265 270

Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
275 280 285

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Thr Glu
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<210> 47
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<212> PRT
<213> Marchantia polymorpha

<400> 47

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Ala Lys Met Lys Gln Ala Arg Ser Ser Pro Glu Gly Glu Ile Val Gly
20 25 30

Gly Asn Arg Met Gly Ser Gly Asn Gly Ala Glu Trp Thr Thr Ser Leu
35 40 45

Ile His Ala Phe Leu Asn Ala Thr Asn Gly Lys Ser Gly Gly Ala Ser
50 55 60

Lys Val Arg Pro Leu Glu Glu Arg Ile Gly Glu Ala Val Phe Arg Val
65 70 75 80

Leu Glu Asp Val Val Gly Val Asp Ile Arg Lys Pro Asn Pro Val Thr
85 90 95

Lys Asp Leu Pro Met Val Glu Ser Pro Val Pro Val Leu Ala Cys Ile
100 105 110

Ser Leu Tyr Leu Leu Val Val Trp Leu Trp Ser Ser His Ile Lys Ala
115 120 125

Ser Gly Gln Lys Pro Arg Lys Glu Asp Pro Leu Ala Leu Arg Cys Leu
130 135 140

Val Ile Ala His Asn Leu Phe Leu Cys Cys Leu Ser Leu Phe Met Cys
145 150 155 160

Val Gly Leu Ile Ala Ala Ala Arg His Tyr Gly Tyr Ser Val Trp Gly
165 170 175

Asn Tyr Tyr Arg Glu Arg Glu Pro Ala Met Asn Leu Leu Ile Tyr Val
180 185 190

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

Phe Arg Arg Asn Leu Arg Gln Val Thr Tyr Leu His Val Tyr His His
210 215 220

20110826_CL4783WOPCT_SequenceListing_ST25

Ala Ser Ile Ala Met Ile Trp Trp Ile Ile Cys Tyr Arg Phe Pro Gly
225 230 235 240

Ala Asp Ser Tyr Phe Ser Ala Ala Phe Asn Ser Cys Ile His Val Ala
245 250 255

Met Tyr Leu Tyr Tyr Leu Leu Ala Ala Thr Val Ala Arg Asp Glu Lys
260 265 270

Arg Arg Arg Lys Tyr Leu Phe Trp Gly Lys Tyr Leu Thr Ile Ile Gln
275 280 285

Met Leu Gln Phe Leu Ser Phe Ile Gly Gln Ala Ile Tyr Ala Met Trp
290 295 300

Lys Phe Glu Tyr Tyr Pro Lys Gly Phe Gly Arg Met Leu Phe Phe Tyr
305 310 315 320

Ser Val Ser Leu Leu Ala Phe Phe Gly Asn Phe Phe Val Lys Lys Tyr
325 330 335

Ser Asn Ala Ser Gln Pro Lys Thr Val Lys Val Glu
340 345

<210> 48
<211> 292
<212> PRT
<213> Ostreococcus tauri
<400> 48

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

Gln Trp Asp Ile Gly Pro Val Ser Ser Ser Thr Ala His Leu Pro Ala
35 40 45

Ile Glu Ser Pro Thr Pro Leu Val Thr Ser Leu Leu Phe Tyr Leu Val
50 55 60

Thr Val Phe Leu Trp Tyr Gly Arg Leu Thr Arg Ser Ser Asp Lys Lys
65 70 75 80

Ile Arg Glu Pro Thr Trp Leu Arg Arg Phe Ile Ile Cys His Asn Ala
85 90 95

Phe Leu Ile Val Leu Ser Leu Tyr Met Cys Leu Gly Cys Val Ala Gln
100 105 110

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Ala Tyr Gln Asn Gly Tyr Thr Leu Trp Gly Asn Glu Phe Lys Ala Thr
115 120 125

Glu Thr Gln Leu Ala Leu Tyr Ile Tyr Ile Phe Tyr Val Ser Lys Ile
130 135 140

Tyr Glu Phe Val Asp Thr Tyr Ile Met Leu Leu Lys Asn Asn Leu Arg
145 150 155 160

Gln Val Ser Phe Leu His Ile Tyr His His Ser Thr Ile Ser Phe Ile
165 170 175

Trp Trp Ile Ile Ala Arg Arg Ala Pro Gly Gly Asp Ala Tyr Phe Ser
180 185 190

Ala Ala Leu Asn Ser Trp Val His Val Cys Met Tyr Thr Tyr Tyr Leu
195 200 205

Leu Ser Thr Leu Ile Gly Lys Glu Asp Pro Lys Arg Ser Asn Tyr Leu
210 215 220

Trp Trp Gly Arg His Leu Thr Gln Met Gln Met Leu Gln Phe Phe Phe
225 230 235 240

Asn Val Leu Gln Ala Leu Tyr Cys Ala Ser Phe Ser Thr Tyr Pro Lys
245 250 255

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

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

Lys Lys Gln Gln
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<210> 49
<211> 277
<212> PRT
<213> Pavlova sp.

<400> 49

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Trp Thr Gly Leu Pro Ile Val Met Ser Val Val Tyr Leu Ser Gly Val
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Phe Gly Leu Thr Lys Tyr Phe Glu Asn Arg Lys Pro Met Thr Gly Leu
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Lys Asp Tyr Met Phe Thr Tyr Asn Leu Tyr Gln Val Ile Ile Asn Val
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Trp Cys Val Val Ala Phe Leu Leu Glu Val Arg Arg Ala Gly Met Ser
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Leu Ile Gly Asn Lys Val Asp Leu Gly Pro Asn Ser Phe Arg Leu Gly
100 105 110

Phe Val Thr Trp Val His Tyr Asn Asn Lys Tyr Val Glu Leu Leu Asp
115 120 125

Thr Leu Trp Met Val Leu Arg Lys Lys Thr Gln Gln Val Ser Phe Leu
130 135 140

His Val Tyr His His Val Leu Leu Met Trp Ala Trp Phe Val Val Val
145 150 155 160

Lys Leu Gly Asn Gly Gly Asp Ala Tyr Phe Gly Gly Leu Met Asn Ser
165 170 175

Ile Ile His Val Met Met Tyr Ser Tyr Tyr Thr Met Ala Leu Leu Gly
180 185 190

Trp Ser Cys Pro Trp Lys Arg Tyr Leu Thr Gln Ala Gln Leu Val Gln
195 200 205

Phe Cys Ile Cys Leu Ala His Ser Thr Trp Ala Ala Val Thr Gly Ala
210 215 220

Tyr Pro Trp Arg Ile Cys Leu Val Glu Val Trp Val Met Val Ser Met
225 230 235 240

Leu Val Leu Phe Thr Arg Phe Tyr Arg Gln Ala Tyr Ala Lys Glu Ala
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Lys Ala Lys Glu Ala Lys Lys Leu Ala Gln Glu Ala Ser Gln Ala Lys
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Ala Val Lys Ala Glu
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<212> PRT

<213> Rebecca salina

<400> 50

Met Lys Ala Ala Ala Gly Lys Val Gln Gln Glu Ala Glu Arg Leu Thr
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 Leu Ser Ala₃₅ Asn Arg Ala Ser Phe₄₀ Tyr Glu Asn Ile Asn₄₅ Asn Glu Lys
 Gly Ala Tyr Ser Thr Ser Trp₅₅ Phe Ser Leu Pro Cys₆₀ Val Met Thr Ala
 Val Tyr Leu Gly Gly Val₇₀ Phe Gly Leu Thr Lys₇₅ Tyr Phe Glu Gly Arg₈₀
 Lys Pro Met Gln Gly₈₅ Leu Lys Asp Tyr Met₉₀ Phe Thr Tyr Asn Leu₉₅ Tyr
 Gln Val Ile Ile₁₀₀ Asn Val Trp Cys Ile₁₀₅ Ala Ala Phe Val Val₁₁₀ Glu Val
 Arg Arg Ala₁₁₅ Gly Met Ser Ala Val₁₂₀ Gly Asn Lys Val Asp₁₂₅ Leu Gly Pro
 Asn Ser₁₃₀ Phe Arg Leu Gly Phe₁₃₅ Val Thr Trp Val His₁₄₀ Tyr Asn Asn Lys
 Tyr Val Glu Leu Leu Asp₁₅₀ Thr Leu Trp Met Val₁₅₅ Leu Arg Lys Lys Thr₁₆₀
 Gln Gln Val Ser Phe₁₆₅ Leu His Val Tyr His₁₇₀ His Val Leu Leu Ile Trp₁₇₅
 Ala Trp Phe Cys₁₈₀ Val Val Lys Phe Cys₁₈₅ Asn Gly Gly Asp Ala₁₉₀ Tyr Phe
 Gly Gly Met₁₉₅ Leu Asn Ser Ile Ile₂₀₀ His Val Met Met Tyr₂₀₅ Ser Tyr Tyr
 Thr Met₂₁₀ Ala Leu Leu Gly Trp₂₁₅ Ser Cys Pro Trp Lys₂₂₀ Arg Tyr Leu Thr
 Gln Ala Gln Leu Val Gln₂₃₀ Phe Cys Ile Cys Leu₂₃₅ Ala His Ala Thr Trp₂₄₀
 Ala Ala Ala Thr Gly₂₄₅ Val Tyr Pro Phe His₂₅₀ Ile Cys Leu Val Glu Ile₂₅₅
 Trp Val Met Val₂₆₀ Ser Met Leu Tyr Leu₂₆₅ Phe Thr Lys Phe Tyr₂₇₀ Asn Ser
 Ala Tyr Lys₂₇₅ Gly Ala Ala Lys Gly₂₈₀ Ala Ala Ala Ser Ser₂₈₅ Asn Gly Ala

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<213> *Ostreococcus tauri*

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35 40 45

Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
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Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
65 70 75 80

Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
85 90 95

Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
100 105 110

Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
115 120 125

Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
130 135 140

Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
145 150 155 160

His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
165 170 175

Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
180 185 190

Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
195 200 205

Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
210 215 220

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Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
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Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
245 250 255

Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
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Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
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<212> PRT
<213> Thalassiosira pseudonana
<400> 52

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His Phe Ala Phe Pro Ala Ala Thr Ala Thr Pro Gly Leu Thr Ala Glu
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Met His Ser Tyr Lys Val Pro Leu Gly Leu Thr Val Phe Tyr Leu Leu
50 55 60

Ser Leu Pro Ser Leu Lys Tyr Val Thr Asp Asn Tyr Leu Ala Lys Lys
65 70 75 80

Tyr Asp Met Lys Ser Leu Leu Thr Glu Ser Met Val Leu Tyr Asn Val
85 90 95

Ala Gln Val Leu Leu Asn Gly Trp Thr Val Tyr Ala Ile Val Asp Ala
100 105 110

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

Ala Ala Leu His Ser Gly Ser Ser Tyr Ala Val Trp Val His Tyr Cys
130 135 140

Asp Lys Tyr Leu Glu Phe Phe Asp Thr Tyr Phe Met Val Leu Arg Gly
145 150 155 160

20110826_CL4783WOPCT_SequenceListing_ST25

Lys Met Asp Gln Val Ser Phe Leu His Ile Tyr His His Thr Thr Ile
165 170 175

Ala Trp Ala Trp Trp Ile Ala Leu Arg Phe Ser Pro Gly Gly Asp Ile
180 185 190

Tyr Phe Gly Ala Leu Leu Asn Ser Ile Ile His Val Leu Met Tyr Ser
195 200 205

Tyr Tyr Ala Leu Ala Leu Leu Lys Val Ser Cys Pro Trp Lys Arg Tyr
210 215 220

Leu Thr Gln Ala Gln Leu Leu Gln Phe Thr Ser Val Val Val Tyr Thr
225 230 235 240

Gly Cys Thr Gly Tyr Thr His Tyr Tyr His Thr Lys His Gly Ala Asp
245 250 255

Glu Thr Gln Pro Ser Leu Gly Thr Tyr Tyr Phe Cys Cys Gly Val Gln
260 265 270

Val Phe Glu Met Val Ser Leu Phe Val Leu Phe Ser Ile Phe Tyr Lys
275 280 285

Arg Ser Tyr Ser Lys Lys Asn Lys Ser Gly Gly Lys Asp Ser Lys Lys
290 295 300

Asn Asp Asp Gly Asn Asn Glu Asp Gln Cys His Lys Ala Met Lys Asp
305 310 315 320

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Arg Val Thr Gly Ala Met
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<213> Thalassiosira pseudonana

<400> 53

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Trp Leu Cys Asp Phe Arg Ser Ala Ile Thr Ile Ala Leu Ile Tyr Ile
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35

40

45

Ala Phe Val Ile Leu Gly Ser Ala Val Met Gln Ser Leu Pro Ala Met
 50 55 60

Asp Pro Tyr Pro Ile Lys Phe Leu Tyr Asn Val Ser Gln Ile Phe Leu
 65 70 75 80

Cys Ala Tyr Met Thr Val Glu Ala Gly Phe Leu Ala Tyr Arg Asn Gly
 85 90 95

Tyr Thr Val Met Pro Cys Asn His Phe Asn Val Asn Asp Pro Pro Val
 100 105 110

Ala Asn Leu Leu Trp Leu Phe Tyr Ile Ser Lys Val Trp Asp Phe Trp
 115 120 125

Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gln Leu Ser Phe
 130 135 140

Leu His Val Tyr His His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
 145 150 155 160

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

Gly Phe Ile His Thr Val Met Tyr Thr Tyr Tyr Phe Ile Cys Met His
 180 185 190

Thr Lys Asp Ser Lys Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser
 195 200 205

Ser Leu Thr Ala Phe Gln Leu Leu Gln Phe Thr Ile Met Met Ser Gln
 210 215 220

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

Thr Ile Val Tyr Phe Val Ser Leu Leu Ser Leu Phe Phe Leu Phe Ala
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Gln Phe Phe Val Gln Ser Tyr Met Ala Pro Lys Lys Lys Lys Ser Ala
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<210> 54

<211> 318

<212> PRT

<213> Mortierella alpina

<400> 54

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

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Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
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Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
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<213> Thraustochytrium sp.

<400> 55

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35 40 45

Leu Val Gly Ser Val Val Leu Tyr Leu Ser Leu Gln Ala Val Val Tyr
50 55 60

Ala Leu Arg Asn Tyr Leu Gly Gly Leu Met Ala Leu Arg Ser Val His
65 70 75 80

Asn Leu Gly Leu Cys Leu Phe Ser Gly Ala Val Trp Ile Tyr Thr Ser
85 90 95

Tyr Leu Met Ile Gln Asp Gly His Phe Arg Ser Leu Glu Ala Ala Thr
100 105 110

Cys Glu Pro Leu Lys His Pro His Phe Gln Leu Ile Ser Leu Leu Phe
115 120 125

Ala Leu Ser Lys Ile Trp Glu Trp Phe Asp Thr Val Leu Leu Ile Val
130 135 140

Lys Gly Asn Lys Leu Arg Phe Leu His Val Leu His His Ala Thr Thr
145 150 155 160

Phe Trp Leu Tyr Ala Ile Asp His Ile Phe Leu Ser Ser Ile Lys Tyr
165 170 175

Gly Val Ala Val Asn Ala Phe Ile His Thr Val Met Tyr Ala His Tyr
180 185 190

Phe Arg Pro Phe Pro Lys Gly Leu Arg Pro Leu Ile Thr Gln Leu Gln
195 200 205

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Ile Val Gln Phe Ile Phe Ser Ile Gly Ile His Thr Ala Ile Tyr Trp
210 215 220

His Tyr Asp Cys Glu Pro Leu Val His Thr His Phe Trp Glu Tyr Val
225 230 235 240

Thr Pro Tyr Leu Phe Val Val Pro Phe Leu Ile Leu Phe Leu Asn Phe
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Tyr Leu Gln Gln Tyr Val Leu Ala Pro Ala Lys Thr Lys Lys Ala
260 265 270

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Lys Val Asp Tyr Ala Gln Leu Trp Ser Asp Ala Ser His Cys Glu Val
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ctg tac ggg tcc atc gcc ttc gtc atc ctg aag ttc acc ctt ggt cct 144

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Leu	Gly	Pro	Lys	Gly	Gln	Ser	Arg	Met	Lys	Phe	Val	Phe	Thr	Asn	Tyr		
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aac	ctg	ctc	atg	tcc	atc	tac	tcg	ctg	ggc	tcc	ttc	ctc	tct	atg	gcc		240
Asn	Leu	Leu	Met	Ser	Ile	Tyr	Ser	Leu	Gly	Ser	Phe	Leu	Ser	Met	Ala		
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tac	gcc	atg	tac	acc	att	ggt	gtc	atg	tcc	gac	aac	tgc	gag	aag	gct		288
Tyr	Ala	Met	Tyr	Thr	Ile	Gly	Val	Met	Ser	Asp	Asn	Cys	Glu	Lys	Ala		
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ttc	gac	aac	aat	gtc	ttc	cga	atc	acc	act	cag	ctg	ttc	tac	ctc	agc		336
Phe	Asp	Asn	Asn	Val	Phe	Arg	Ile	Thr	Thr	Gln	Leu	Phe	Tyr	Leu	Ser		
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aag	ttc	ctc	gag	tac	att	gac	tcc	ttc	tat	ctg	ccc	ctc	atg	ggc	aag		384
Lys	Phe	Leu	Glu	Tyr	Ile	Asp	Ser	Phe	Tyr	Leu	Pro	Leu	Met	Gly	Lys		
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Pro	Leu	Thr	Trp	Leu	Gln	Phe	Phe	His	His	Leu	Gly	Ala	Pro	Met	Asp		
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Met	Trp	Leu	Phe	Tyr	Asn	Tyr	Arg	Asn	Glu	Ala	Val	Trp	Ile	Phe	Val		
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ctg	ctc	aac	ggc	ttc	att	cac	tgg	atc	atg	tac	ggc	tac	tat	tgg	acc		528
Leu	Leu	Asn	Gly	Phe	Ile	His	Trp	Ile	Met	Tyr	Gly	Tyr	Tyr	Trp	Thr		
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cga	ctg	atc	aag	ctc	aag	ttc	cct	atg	ccc	aag	tcc	ctg	att	act	tct		576
Arg	Leu	Ile	Lys	Leu	Lys	Phe	Pro	Met	Pro	Lys	Ser	Leu	Ile	Thr	Ser		
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Met	Gln	Ile	Ile	Gln	Phe	Asn	Val	Gly	Phe	Tyr	Ile	Val	Trp	Lys	Tyr		
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Arg	Asn	Ile	Pro	Cys	Tyr	Arg	Gln	Asp	Gly	Met	Arg	Met	Phe	Gly	Trp		
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Phe	Phe	Asn	Tyr	Phe	Tyr	Val	Gly	Thr	Val	Leu	Cys	Leu	Phe	Leu	Asn		
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ttc	tac	gtg	cag	acc	tac	atc	gtc	cga	aag	cac	aag	gga	gcc	aaa	aag		768
Phe	Tyr	Val	Gln	Thr	Tyr	Ile	Val	Arg	Lys	His	Lys	Gly	Ala	Lys	Lys		
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Ile	Gln																

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<212> PRT

<213> Euglena gracilis

<400> 59

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Ile	Gln																		

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<212> DNA
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Lys	Val	Asp	Tyr	Ala	Gln	Leu	Trp	Ser	Asp	Ala	Ser	His	Cys	Glu	Val		
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Asn	Leu	Leu	Met	Ser	Ile	Tyr	Ser	Leu	Gly	Ser	Phe	Leu	Ser	Met	Ala		
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Tyr	Ala	Met	Tyr	Thr	Ile	Gly	Val	Met	Ser	Asp	Asn	Cys	Glu	Lys	Ala		
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Lys	Phe	Leu	Glu	Tyr	Ile	Asp	Ser	Phe	Tyr	Leu	Pro	Leu	Met	Gly	Lys		
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Arg	Leu	Ile	Lys	Leu	Lys	Phe	Pro	Met	Pro	Lys	Ser	Leu	Ile	Thr	Ser		
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Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
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Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
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Tyr Ala Met Tyr Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
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Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Glu Leu Phe Tyr Leu Ser
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Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
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<211> 777

<212> DNA

<213> Euglena gracilis

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<223> mutant delta-9 elongase "EgD9eS-A21V/L35G/L108G/I179R"

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48

aag gtc gac tat gtg cag ctc tgg tct gat gcc tcg cac tgc gag gtg
Lys Val Asp Tyr Val Gln Leu Trp Ser Asp Ala Ser His Cys Glu Val
20 25 30

96

ctg tac ggg tcc atc gcc ttc gtc atc ctg aag ttc acc ctt ggt cct
Leu Tyr Gly Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro
35 40 45

144

ctc gga ccc aag ggt cag tct cga atg aag ttt gtg ttc acc aac tac
Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
50 55 60

192

aac ctg ctc atg tcc atc tac tcg ctg ggc tcc ttc ctc tct atg gcc
Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
65 70 75 80

240

tac gcc atg tac acc att ggt gtc atg tcc gac aac tgc gag aag gct
Tyr Ala Met Tyr Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
85 90 95

288

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Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Gly Phe Tyr Leu Ser
100 105 110

336

aag ttc ctc gag tac att gac tcc ttc tat ctg ccc ctc atg ggc aag
Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
115 120 125

384

cct ctg acc tgg ttg cag ttc ttt cac cat ctc gga gct cct atg gac
Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
130 135 140

432

atg tgg ctg ttc tac aac tac cga aac gaa gcc gtt tgg atc ttt gtg
Met Trp Leu Phe Tyr Asn Tyr Arg Asn Glu Ala Val Trp Ile Phe Val
145 150 155 160

480

ctg ctc aac ggc ttc att cac tgg atc atg tac ggc tac tat tgg acc
Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
165 170 175

528

cga ctg cga aag ctc aag ttc cct atg ccc aag tcc ctg att act tct
Arg Leu Arg Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

576

atg cag atc att cag ttc aac gtt ggc ttc tac atc gtc tgg aag tac

624

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Arg	Asn	Ile	Pro	Cys	Tyr	Arg	Gln	Asp	Gly	Met	Arg	Met	Phe	Gly	Trp		
	210					215					220						
ttt	ttc	aac	tac	ttc	tac	gtt	ggg	act	gtc	ctg	tgt	ctg	ttc	ctc	aac		720
Phe	Phe	Asn	Tyr	Phe	Tyr	Val	Gly	Thr	Val	Leu	Cys	Leu	Phe	Leu	Asn		
225					230					235					240		
ttc	tac	gtg	cag	acc	tac	atc	gtc	cga	aag	cac	aag	gga	gcc	aaa	aag		768
Phe	Tyr	Val	Gln	Thr	Tyr	Ile	Val	Arg	Lys	His	Lys	Gly	Ala	Lys	Lys		
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att	cag	tga															777
Ile	Gln																

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<400> 87

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			20					25					30				
Leu	Tyr	Gly	Ser	Ile	Ala	Phe	Val	Ile	Leu	Lys	Phe	Thr	Leu	Gly	Pro		
		35					40					45					
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	50					55					60						
Asn	Leu	Leu	Met	Ser	Ile	Tyr	Ser	Leu	Gly	Ser	Phe	Leu	Ser	Met	Ala		
65					70				75						80		
Tyr	Ala	Met	Tyr	Thr	Ile	Gly	Val	Met	Ser	Asp	Asn	Cys	Glu	Lys	Ala		
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Phe	Asp	Asn	Asn	Val	Phe	Arg	Ile	Thr	Thr	Gln	Gly	Phe	Tyr	Leu	Ser		
			100					105					110				
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Pro	Leu	Thr	Trp	Leu	Gln	Phe	Phe	His	His	Leu	Gly	Ala	Pro	Met	Asp		
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Arg Leu Arg Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
195 200 205

Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
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Ile Gln

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Phe Ile Leu Val Ile Ala Leu Pro Leu Ser Ser Phe Ala Ala Ala Pro	
65 70 75 80	
ttc gtc tcc ttc aac tgg aag acc gcc gcg ttt gct gtc ggc tat tac	288
Phe Val Ser Phe Asn Trp Lys Thr Ala Ala Phe Ala Val Gly Tyr Tyr	
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His	Arg	Ala	Tyr	Lys	Ala	Ala	Leu	Pro	Val	Arg	Ile	Ile	Leu	Ala	Leu	
		115					120					125				
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Phe	Gly	Gly	Gly	Ala	Val	Glu	Gly	Ser	Ile	Arg	Trp	Trp	Ala	Ser	Ser	
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cac	cga	gtc	cac	cac	cga	tgg	acc	gac	tcc	aac	aag	gac	cct	tac	gac	480
His	Arg	Val	His	His	Arg	Trp	Thr	Asp	Ser	Asn	Lys	Asp	Pro	Tyr	Asp	
					150					155					160	
gcc	cga	aag	gga	ttc	tgg	ttc	tcc	cac	ttt	ggc	tgg	atg	ctg	ctt	gtg	528
Ala	Arg	Lys	Gly	Phe	Trp	Phe	Ser	His	Phe	Gly	Trp	Met	Leu	Leu	Val	
				165					170					175		
ccc	aac	ccc	aag	aac	aag	ggc	cga	act	gac	att	tct	gac	ctc	aac	aac	576
Pro	Asn	Pro	Lys	Asn	Lys	Gly	Arg	Thr	Asp	Ile	Ser	Asp	Leu	Asn	Asn	
			180					185					190			
gac	tgg	gtt	gtc	cga	ctc	cag	cac	aag	tac	tac	gtt	tac	gtt	ctc	gtc	624
Asp	Trp	Val	Val	Arg	Leu	Gln	His	Lys	Tyr	Tyr	Val	Tyr	Val	Leu	Val	
		195					200					205				
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Phe	Met	Ala	Ile	Val	Leu	Pro	Thr	Leu	Val	Cys	Gly	Phe	Gly	Trp	Gly	
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Asp	Trp	Lys	Gly	Gly	Leu	Val	Tyr	Ala	Gly	Ile	Met	Arg	Tyr	Thr	Phe	
					230					235					240	
gtg	cag	cag	gtg	act	ttc	tgt	gtc	aac	tcc	ctt	gcc	cac	tgg	att	gga	768
Val	Gln	Gln	Val	Thr	Phe	Cys	Val	Asn	Ser	Leu	Ala	His	Trp	Ile	Gly	
				245					250					255		
gag	cag	ccc	ttc	gac	gac	cga	cga	act	ccc	cga	gac	cac	gct	ctt	acc	816
Glu	Gln	Pro	Phe	Asp	Asp	Arg	Arg	Thr	Pro	Arg	Asp	His	Ala	Leu	Thr	
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Ala	Leu	Val	Thr	Phe	Gly	Glu	Gly	Tyr	His	Asn	Phe	His	His	Glu	Phe	
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Pro	Ser	Asp	Tyr	Arg	Asn	Ala	Leu	Ile	Trp	Tyr	Gln	Tyr	Asp	Pro	Thr	
	290					295					300					
aag	tgg	ctc	atc	tgg	acc	ctc	aag	cag	gtt	ggt	ctc	gcc	tgg	gac	ctc	960
Lys	Trp	Leu	Ile	Trp	Thr	Leu	Lys	Gln	Val	Gly	Leu	Ala	Trp	Asp	Leu	
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cag	acc	ttc	tcc	cag	aac	gcc	atc	gag	cag	ggt	ctc	gtg	cag	cag	cga	1008
Gln	Thr	Phe	Ser	Gln	Asn	Ala	Ile	Glu	Gln	Gly	Leu	Val	Gln	Gln	Arg	
				325					330					335		
cag	aag	aag	ctg	gac	aag	tgg	cga	aac	aac	ctc	aac	tgg	ggt	atc	ccc	1056
Gln	Lys	Lys	Leu	Asp	Lys	Trp	Arg	Asn	Asn	Leu	Asn	Trp	Gly	Ile	Pro	
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att	gag	cag	ctg	cct	gtc	att	gag	ttt	gag	gag	ttc	caa	gag	cag	gcc	1104
Ile	Glu	Gln	Leu	Pro	Val	Ile	Glu	Phe	Glu	Glu	Phe	Gln	Glu	Gln	Ala	
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370						375					380						
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Ala	Phe	Val	Glu	His	His	Pro	Gly	Gly	Lys	Ala	Leu	Ile	Met	Ser	Ala		
385					390					395					400		
gtc	ggc	aag	gac	ggt	acc	gct	gtc	ttc	aac	gga	ggt	gtc	tac	cga	cac	1248	
Val	Gly	Lys	Asp	Gly	Thr	Ala	Val	Phe	Asn	Gly	Gly	Val	Tyr	Arg	His		
				405					410					415			
tcc	aac	gct	ggc	cac	aac	ctg	ctt	gcc	acc	atg	cga	gtt	tcg	gtc	att	1296	
Ser	Asn	Ala	Gly	His	Asn	Leu	Leu	Ala	Thr	Met	Arg	Val	Ser	Val	Ile		
			420					425					430				
cga	ggc	ggc	atg	gag	gtt	gag	gtg	tgg	aag	act	gcc	cag	aac	gaa	aag	1344	
Arg	Gly	Gly	Met	Glu	Val	Glu	Val	Trp	Lys	Thr	Ala	Gln	Asn	Glu	Lys		
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aag	gac	cag	aac	att	gtc	tcc	gat	gag	agt	gga	aac	cga	atc	cac	cga	1392	
Lys	Asp	Gln	Asn	Ile	Val	Ser	Asp	Glu	Ser	Gly	Asn	Arg	Ile	His	Arg		
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gct	ggt	ctc	cag	gcc	acc	cgg	gtc	gag	aac	ccc	ggt	atg	tct	ggc	atg	1440	
Ala	Gly	Leu	Gln	Ala	Thr	Arg	Val	Glu	Asn	Pro	Gly	Met	Ser	Gly	Met		
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Ala	Ala																

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<400> 92

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			20					25					30		

Lys	Met	Ser	Gln	Gly	Ala	Tyr	Asp	Asp	Lys	Gly	Arg	His	Ile	Ser	Glu
		35					40					45			

Gln	Pro	Phe	Thr	Trp	Ala	Asn	Trp	His	Gln	His	Ile	Asn	Trp	Leu	Asn
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Phe	Ile	Leu	Val	Ile	Ala	Leu	Pro	Leu	Ser	Ser	Phe	Ala	Ala	Ala	Pro
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Phe	Val	Ser	Phe	Asn	Trp	Lys	Thr	Ala	Ala	Phe	Ala	Val	Gly	Tyr	Tyr
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Met	Cys	Thr	Gly	Leu	Gly	Ile	Thr	Ala	Gly	Tyr	His	Arg	Met	Trp	Ala
			100					105					110		

His	Arg	Ala	Tyr	Lys	Ala	Ala	Leu	Pro	Val	Arg	Ile	Ile	Leu	Ala	Leu
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115

120

125

Phe Gly Gly Gly Ala Val Glu Gly Ser Ile Arg Trp Trp Ala Ser Ser
 130 135 140

His Arg Val His His Arg Trp Thr Asp Ser Asn Lys Asp Pro Tyr Asp
 145 150 155 160

Ala Arg Lys Gly Phe Trp Phe Ser His Phe Gly Trp Met Leu Leu Val
 165 170 175

Pro Asn Pro Lys Asn Lys Gly Arg Thr Asp Ile Ser Asp Leu Asn Asn
 180 185 190

Asp Trp Val Val Arg Leu Gln His Lys Tyr Tyr Val Tyr Val Leu Val
 195 200 205

Phe Met Ala Ile Val Leu Pro Thr Leu Val Cys Gly Phe Gly Trp Gly
 210 215 220

Asp Trp Lys Gly Gly Leu Val Tyr Ala Gly Ile Met Arg Tyr Thr Phe
 225 230 235 240

Val Gln Gln Val Thr Phe Cys Val Asn Ser Leu Ala His Trp Ile Gly
 245 250 255

Glu Gln Pro Phe Asp Asp Arg Arg Thr Pro Arg Asp His Ala Leu Thr
 260 265 270

Ala Leu Val Thr Phe Gly Glu Gly Tyr His Asn Phe His His Glu Phe
 275 280 285

Pro Ser Asp Tyr Arg Asn Ala Leu Ile Trp Tyr Gln Tyr Asp Pro Thr
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Lys Trp Leu Ile Trp Thr Leu Lys Gln Val Gly Leu Ala Trp Asp Leu
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Gln Thr Phe Ser Gln Asn Ala Ile Glu Gln Gly Leu Val Gln Gln Arg
 325 330 335

Gln Lys Lys Leu Asp Lys Trp Arg Asn Asn Leu Asn Trp Gly Ile Pro
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Ile Glu Gln Leu Pro Val Ile Glu Phe Glu Glu Phe Gln Glu Gln Ala
 355 360 365

Lys Thr Arg Asp Leu Val Leu Ile Ser Gly Ile Val His Asp Val Ser
 370 375 380

Ala Phe Val Glu His His Pro Gly Gly Lys Ala Leu Ile Met Ser Ala
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385 390 395 400

Val Gly Lys Asp Gly Thr Ala Val Phe Asn Gly Gly Val Tyr Arg His
405 410 415

Ser Asn Ala Gly His Asn Leu Leu Ala Thr Met Arg Val Ser Val Ile
420 425 430

Arg Gly Gly Met Glu Val Glu Val Trp Lys Thr Ala Gln Asn Glu Lys
435 440 445

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Gly Ser Asp Glu Gly Leu Thr Asp Thr Ser Gly His Val Ser Pro Ala
20 25 30
gcc aag aag cag aag aac tcg gag att cat ttc acc acc cag gct gcc 144
Ala Lys Lys Gln Lys Asn Ser Glu Ile His Phe Thr Thr Gln Ala Ala
35 40 45
cag cag ttg gat cgg gag cgc aag gag gag tat ctg gac tcg ctg atc 192
Gln Gln Leu Asp Arg Glu Arg Lys Glu Glu Tyr Leu Asp Ser Leu Ile
50 55 60
gac aac aag gac tat ctc aag tac cgt cct cga ggc tgg aag ctc aac 240
Asp Asn Lys Asp Tyr Leu Lys Tyr Arg Pro Arg Gly Trp Lys Leu Asn
65 70 75 80
aac ccg cct acc gac cga cct gtg cga atc tac gcc gat gga gtg ttt 288
Asn Pro Pro Thr Asp Arg Pro Val Arg Ile Tyr Ala Asp Gly Val Phe
85 90 95
gat ttg ttc cat ctg gga cac atg cgt cag ctg gag cag tcc aag aag 336
Asp Leu Phe His Leu Gly His Met Arg Gln Leu Glu Gln Ser Lys Lys
100 105 110

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acc	cac	aag	cgg	aag	gga	ttg	acc	gtg	ctg	agt	gac	gtc	cag	cgg	tac	432
Thr	His	Lys	Arg	Lys	Gly	Leu	Thr	Val	Leu	Ser	Asp	Val	Gln	Arg	Tyr	
	130					135					140					
gag	acg	gtg	cga	cac	tgc	aag	tgg	gtg	gac	gag	gtg	gtg	gag	gat	gct	480
Glu	Thr	Val	Arg	His	Cys	Lys	Trp	Val	Asp	Glu	Val	Val	Glu	Asp	Ala	
145					150					155					160	
ccc	tgg	tgt	gtc	acc	atg	gac	ttt	ctg	gaa	aaa	cac	aaa	atc	gac	tac	528
Pro	Trp	Cys	Val	Thr	Met	Asp	Phe	Leu	Glu	Lys	His	Lys	Ile	Asp	Tyr	
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gtg	gcc	cat	gac	gat	ctg	ccc	tac	gct	tcc	ggc	aac	gac	gat	gat	atc	576
Val	Ala	His	Asp	Asp	Leu	Pro	Tyr	Ala	Ser	Gly	Asn	Asp	Asp	Asp	Ile	
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Tyr	Lys	Pro	Ile	Lys	Glu	Lys	Gly	Met	Phe	Leu	Ala	Thr	Gln	Arg	Thr	
		195					200					205				
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Glu	Gly	Ile	Ser	Thr	Ser	Asp	Ile	Ile	Thr	Lys	Ile	Ile	Arg	Asp	Tyr	
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Asp	Lys	Tyr	Leu	Met	Arg	Asn	Phe	Ala	Arg	Gly	Ala	Asn	Arg	Lys	Asp	
225				230						235					240	
ctc	aac	gtc	tcg	tgg	ctc	aag	aag	aac	gag	ctg	gac	ttc	aag	cgt	cat	768
Leu	Asn	Val	Ser	Trp	Leu	Lys	Lys	Asn	Glu	Leu	Asp	Phe	Lys	Arg	His	
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gtg	gcc	gag	ttc	cga	aac	tcg	ttc	aag	cga	aag	aag	gtc	ggt	aag	gat	816
Val	Ala	Glu	Phe	Arg	Asn	Ser	Phe	Lys	Arg	Lys	Lys	Val	Gly	Lys	Asp	
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Leu	Tyr	Gly	Glu	Ile	Arg	Gly	Leu	Leu	Gln	Asn	Val	Leu	Ile	Trp	Asn	
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305					310					315					320	
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Asp	Ala	Val	Asp	Val	Asp	Ser	Ser	Glu	Asn	Val	Ser	Glu	Asn	Val	Thr	
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gat	gag	gag	gag	gaa	gac	gac	gac	gag	ggt	gat	gag	gac	gaa	gaa	gcc	1056
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 Asp Asn Lys Asp Tyr Leu Lys Tyr Arg Pro Arg Gly Trp Lys Leu Asn
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 Asn Pro Pro Thr Asp Arg Pro Val Arg Ile Tyr Ala Asp Gly Val Phe
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 Asp Leu Phe His Leu Gly His Met Arg Gln Leu Glu Gln Ser Lys Lys
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 Ala Phe Pro Asn Ala Val Leu Ile Val Gly Ile Pro Ser Asp Lys Glu
 115 120 125
 Thr His Lys Arg Lys Gly Leu Thr Val Leu Ser Asp Val Gln Arg Tyr
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 Glu Thr Val Arg His Cys Lys Trp Val Asp Glu Val Val Glu Asp Ala
 145 150 155 160
 Pro Trp Cys Val Thr Met Asp Phe Leu Glu Lys His Lys Ile Asp Tyr
 165 170 175
 Val Ala His Asp Asp Leu Pro Tyr Ala Ser Gly Asn Asp Asp Asp Ile
 180 185 190
 Tyr Lys Pro Ile Lys Glu Lys Gly Met Phe Leu Ala Thr Gln Arg Thr
 195 200 205
 Glu Gly Ile Ser Thr Ser Asp Ile Ile Thr Lys Ile Ile Arg Asp Tyr
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 Asp Lys Tyr Leu Met Arg Asn Phe Ala Arg Gly Ala Asn Arg Lys Asp
 225 230 235 240
 Leu Asn Val Ser Trp Leu Lys Lys Asn Glu Leu Asp Phe Lys Arg His
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Leu Tyr Gly Glu Ile Arg Gly Leu Leu Gln Asn Val Leu Ile Trp Asn
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Gly Asp Asn Ser Gly Thr Ser Thr Pro Gln Arg Lys Thr Leu Gln Thr
290 295 300

Asn Ala Lys Lys Met Tyr Met Asn Val Leu Lys Thr Leu Gln Ala Pro
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 atcctgaagt tcacccttgg tcctctcgga cccaagggtc agtctcgaat gaagtttgtg 180
 ttcaccaact acaacctgct catgtccatc tactcgctgg gtccttcct ctctatggcc 240
 tacgccatgt acaccattgg tgtcatgtcc gacaactgcg agaaggcttt cgacaacaat 300
 gtcttccgaa tcaccactca gctgttctac ctacagcaagt tcctcgagta cattgactcc 360
 ttctatctgc ccctcatggg caagcctctg acctgggtgc agttctttca ccatctcgga 420
 gtcctatgg acatgtggct gttctacaac taccgaaacg aagccgtttg gatctttgtg 480
 ctgctcaacg gcttcattca ctggatcatg tacggctact attggaccg actgatcaag 540

20110826_CL4783WOPCT_SequenceListing_ST25

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 Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
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 65 70 75 80

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Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys	
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ctg	ctc	aac	ggc	ttc	att	cac	tgg	atc	atg	tac	ggc	tac	tat	tgg	acc	528
Leu	Leu	Asn	Gly	Phe	Ile	His	Trp	Ile	Met	Tyr	Gly	Tyr	Tyr	Trp	Thr	
			165						170					175		
cga	ctg	cga	aag	ctc	aag	ttc	cct	atg	ccc	aag	tcc	ctg	att	act	tct	576
Arg	Leu	Arg	Lys	Leu	Lys	Phe	Pro	Met	Pro	Lys	Ser	Leu	Ile	Thr	Ser	
			180					185					190			
atg	cag	atc	att	cag	ttc	aac	gtt	ggc	ttc	tac	atc	gtc	tgg	aag	tac	624
Met	Gln	Ile	Ile	Gln	Phe	Asn	Val	Gly	Phe	Tyr	Ile	Val	Trp	Lys	Tyr	
		195					200					205				
cgg	aac	att	ccc	tgc	tac	cga	caa	gat	gga	atg	aga	atg	ttt	ggc	tgg	672
Arg	Asn	Ile	Pro	Cys	Tyr	Arg	Gln	Asp	Gly	Met	Arg	Met	Phe	Gly	Trp	
	210					215					220					
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Phe	Phe	Asn	Tyr	Phe	Tyr	Val	Gly	Thr	Val	Leu	Cys	Leu	Phe	Leu	Asn	
225				230					235						240	
ttc	tac	gtg	cag	acc	tac	atc	gtc	cga	aag	cac	aag	gga	gcc	aaa	aag	768
Phe	Tyr	Val	Gln	Thr	Tyr	Ile	Val	Arg	Lys	His	Lys	Gly	Ala	Lys	Lys	
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Ile	Gln															

<210> 104
 <211> 258
 <212> PRT
 <213> Euglena gracilis

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<400> 104

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35 40 45

Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
50 55 60

Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
65 70 75 80

Tyr Ala Met Cys Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
85 90 95

Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
100 105 110

Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
115 120 125

Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
130 135 140

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

Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
165 170 175

Arg Leu Arg Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr
195 200 205

Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
225 230 235 240

Phe Tyr Val Gln Thr Tyr Ile Val Arg Lys His Lys Gly Ala Lys Lys
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Ile Gln

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<210> 105
 <211> 7769
 <212> DNA
 <213> Artificial Sequence

<220>
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cactgcataa ttctcttact gtcatgccat ccgtaagatg cttttctgtg actggtgagt	1980
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20110826_CL4783WOPCT_SequenceListing_ST25

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 <211> 777
 <212> DNA
 <213> Euglena gracilis

<220>
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aag gtc gac tat gct cag ctc tgg tct gat gcc tcg cac tgc gag gtg	96
Lys Val Asp Tyr Ala Gln Leu Trp Ser Asp Ala Ser His Cys Glu Val	
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ctg tac ggg tcc atc gcc ttc gtc atc ctg aag ttc acc ctt ggt cct	144
Leu Tyr Gly Ser Ile Ala Phe Val Ile Leu Lys Phe Thr Leu Gly Pro	
35 40 45	
ctc gga ccc aag ggt cag tct cga atg aag ttt gtg ttc acc aac tac	192
Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr	
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aac ctg ctc atg tcc atc tac tcg ctg ggc tcc ttc ctc tct atg gcc	240
Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala	
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Tyr Ala Met Cys Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala	
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Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser	
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Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys	
115 120 125	
cct ctg acc tgg ttg cag ttc ttt cac cat ctc gga gct cct atg gac	432
Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp	
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Met Trp Leu Phe Tyr Asn Tyr Arg Asn Glu Ala Val Trp Ile Phe Val	
145 150 155 160	
ctg ctc aac ggc ttc att cac tgg atc atg tac ggc tac tat tgg acc	528
Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr	
165 170 175	
cga ctg cga aag ctc aag ttc cct atg ccc aag tcc ctg att act tct	576
Arg Leu Arg Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser	
180 185 190	
atg cag atc att cag ttc aac gtt ggc ttc tac atc gtc tgg aag tac	624
Met Gln Ile Ile Gln Phe Asn Val Gly Phe Tyr Ile Val Trp Lys Tyr	
195 200 205	
cgg aac att ccc tgc tac cga caa gat gga atg aga atg ttt ggc tgg	672
Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp	
210 215 220	

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225 230 235 240

ttc tac gtg aac acc tac atc gtc cga aag cac aag gga gcc aaa aag 768
Phe Tyr Val Asn Thr Tyr Ile Val Arg Lys His Lys Gly Ala Lys Lys
245 250 255

att cag tga 777
Ile Gln

<210> 107
<211> 258
<212> PRT
<213> Euglena gracilis
<400> 107

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35 40 45

Leu Gly Pro Lys Gly Gln Ser Arg Met Lys Phe Val Phe Thr Asn Tyr
50 55 60

Asn Leu Leu Met Ser Ile Tyr Ser Leu Gly Ser Phe Leu Ser Met Ala
65 70 75 80

Tyr Ala Met Cys Thr Ile Gly Val Met Ser Asp Asn Cys Glu Lys Ala
85 90 95

Phe Asp Asn Asn Val Phe Arg Ile Thr Thr Gln Leu Phe Tyr Leu Ser
100 105 110

Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro Leu Met Gly Lys
115 120 125

Pro Leu Thr Trp Leu Gln Phe Phe His His Leu Gly Ala Pro Met Asp
130 135 140

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

Leu Leu Asn Gly Phe Ile His Trp Ile Met Tyr Gly Tyr Tyr Trp Thr
165 170 175

Arg Leu Arg Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

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195 200 205

Arg Asn Ile Pro Cys Tyr Arg Gln Asp Gly Met Arg Met Phe Gly Trp
210 215 220

Phe Phe Asn Tyr Phe Tyr Val Gly Thr Val Leu Cys Leu Phe Leu Asn
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245 250 255

Ile Gln

<210> 108
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<212> DNA
<213> Artificial Sequence

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115

120

125

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Arg Leu Arg Lys Leu Lys Phe Pro Met Pro Lys Ser Leu Ile Thr Ser
180 185 190

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210 215 220

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