(19) DANMARK

(10) DK/EP 2861728 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 12 N 9/10 (2006.01)* *C 12 P 7/64 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2018-03-26**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2017-12-27**

(86) Europæisk ansøgning nr.: **13731233.6**

(86) Europæisk indleveringsdag: **2013-06-13**

(87) Den europæiske ansøgnings publiceringsdag: **2015-04-22**

(86) International ansøgning nr.: **US2013045592**

(87) Internationalt publikationsnr.: **WO2013192002**

(30) Prioritet: **2012-06-19 US 201261661623 P** **2012-06-19 US 201261661615 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **FORBEDRET FREMSTILLING AF POLYUMÆTTEDE FEDTSYRER VED COEKSPRESSION AF ACYL-COA:LYSOPHOSPHATIDYLCHOLINACYLTRANSFERASER OG PHOSPHOLIPID:DIACYLGLYCEROLACYLTRANSFERASER**

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DESCRIPTION

[0001] This application claims the benefit of U.S. Provisional Application Nos. 61/661,615 and 61/661,623, each filed June 19, 2012.

FIELD OF THE INVENTION

[0002] This invention is in the field of biotechnology. More specifically, this invention pertains to over-expression of both a polynucleotide sequence encoding acyl-CoA:lysophosphatidylcholine acyltransferase and a polynucleotide sequence encoding phospholipid:diacylglycerol acyltransferase as a means to improve production of long-chain polyunsaturated fatty acids ["PUFAs"] in a recombinant microbial cell.

BACKGROUND OF THE INVENTION

[0003] Glycerophospholipids, the main component of biological membranes, contain a glycerol core with fatty acids attached as R groups at the *sn*-1 position and *sn*-2 position, and a polar head group joined at the *sn*-3 position via a phosphodiester bond. The specific polar head group determines the name given to a particular glycerophospholipid (e.g., a chloline head group results in a phosphatidylcholine). Glycerophospholipids possess tremendous diversity, not only resulting from variable phosphoryl head groups, but also as a result of differing chain lengths and degrees of saturation of their fatty acids. Generally, saturated and monounsaturated fatty acids are esterified at the *sn*-1 position, while polyunsaturated fatty acids are esterified at the *sn-2* position.

[0004] Glycerophospholipid biosynthesis, summarized in U.S. Pat. Appl. Publ. No. 2010-0317882-A1, requires a variety of acyltransferases, including glycerol-3-phosphate acyltransferase (GPAT) [E.C. 2.3.1.15], acyl-CoA:lysophosphatidic acid acyltransferase (LPAAT) [E.C. 2.3.1.51], diacylglycerol acyltransferase (DGAT) [E.C. 2.3.1.20] and phospholipid:diacylglycerol acyltransferase (PDAT) [E.C.2.3.1.158].

[0005] Following their *de novo* synthesis, glycerophospholipids can undergo rapid turnover of the fatty acyl composition at the *sn*-2 position. This "remodeling", or "acyl editing", is important for membrane structure and function, biological response to stress conditions, and manipulation of fatty acid composition and quantity in biotechnological applications. Specifically, the remodeling has been attributed to a combination of deacylation and reacylation of glycerophospholipid. For example, in the Lands' cycle (Lands, J. Biol. Chem., 231:883-888 (1958)), remodeling occurs through the concerted action of: 1) a phospholipase, such as phospholipase A_2, that releases fatty acids from the *sn-2* position of phosphatidylcholine; and 2) acyl-CoA:lysophospholipid acyltransferases ["LPLATs"], such as acyl-CoA:lysophosphatidylcholine acyltransferase ["LPCAT"] that reacylates the lysophosphatidylcholine ["LPC"] at the *sn-2* position (thereby removing acyl-CoA fatty acids from the cellular acyl-CoA pool and acylating lysophospholipid substrates at the *sn-2* position in the phospholipid pool). Remodeling has also been attributed to reversible LPCAT activity (Stymne and Stobart (Biochem J., 223(2):305-314(1984))

[0006] The effect of LPCATs (and other LPLATs that have LPCAT activity) on polyunsaturated fatty acid ["PUFA"] production has been contemplated, since fatty acid biosynthesis requires rapid exchange of acyl groups between the acyl-CoA pool and the phospholipid pool. Specifically, desaturations occur mainly at the *sn-2* position of phospholipids, while elongation occurs in the acyl-CoA pool. More specifically, U.S. Pat. No. 7,932,077 hypothesized that acyltransferases, including PDAT and LPCAT, could be important in the accumulation of PUFAs (e.g., eicosapentaenoic acid ["EPA"], 20:5 omega-3) in the TAG fraction of *Yarrowia lipolytica*. As described therein, this was based on the following studies: 1) Stymne and Stobart (Biochem J., 223(2):305-314(1984)), who hypothesized that the exchange between the acyl-CoA pool and PC pool may be attributed to the forward and backward reaction of LPCAT; 2) Domergue et al. (J. Biol. Chem., 278:35115-35126 (2003)), who suggested that accumulation of gamma-linolenic acid ["GLA"] at the *sn*-2 position of phosphatidylcholine ["PC"] and the inability to efficiently synthesize arachidonic acid ["ARA"] (20:4 omega-6) in yeast was a result of the elongation step involved in PUFA biosynthesis occurring within the acyl-CoA pool, while delta-5 and delta-6 desaturation steps occurred predominantly at the *sn-2* position of PC; 3) Abbadi et al. (The Plant Cell, 16:2734-2748 (2004)), who suggested that LPCAT plays a critical role in the successful reconstitution of a delta-6 desaturase/delta-6 elongase pathway, based on analysis of the constraints of PUFA accumulation in transgenic oilseed plants; and 4) Intl. Appl. Publ. No. WO 2004/076617 A2 (Renz et al.), who provided a gene encoding LPCAT from *Caenorhabditis elegans* (T06E8.1) that substantially improved the efficiency of elongation in a genetically introduced delta-6 desaturase/delta-6 elongase pathway in S. *cerevisiae* fed with exogenous fatty acid substrates suitable for delta-6 elongation. Renz et al. concluded that LPCAT allowed efficient and continuous exchange of the newly synthesized fatty acids between phospholipids and the acyl-CoA pool, since desaturases catalyze the introduction of double bonds in PC-coupled fatty acids while elongases exclusively catalyze the elongation of CoA-esterified fatty acids (acyl-CoA).

[0007] U.S. Pat. Appl. Publ. No. 2010-0317882-A1 provided further support that LPCAT is indeed important in the accumulation of

EPA and docosahexaenoic acid ["DHA"] (22:6 omega-3) in the TAG fraction of *Yarrowia lipolytica*. It was found that over-expression of LPCATs can result in an improvement in the delta-9 elongase conversion efficiency and/or delta-4 desaturase conversion efficiency (wherein conversion efficiency is a term that refers to the efficiency by which a particular enzyme can convert substrate to product). Thus, in a strain engineered to produce EPA, improvement in delta-9 elongase conversion efficiency was demonstrated to result in increased EPA % TFAs or EPA % DCW. Similarly, improvement in delta-9 elongase and/or delta-4 desaturase conversion efficiency in a strain engineered to produce DHA was demonstrated to result in increased DHA % TFAs or DHA % DCW.

[0008] Numerous other references generally describe benefits of co-expressing LPLATs with PUFA biosynthetic genes to increase the amount of a desired fatty acid in the oil of a transgenic organism, increase total oil content, or selectively increase the content of desired fatty acids (e.g., Intl. Appl. Publication Nos. WO 2004/087902, WO 2006/069936, WO 2006/052870, WO 2009/001315, WO 2009/014140). However, none of these references describe the benefits achieved in an organism engineered for high-level production of LC-PUFAs when an LPCAT and a phospholipid:diacylglycerol acyltransferase (PDAT) are both over-expressed. PDAT is an enzyme responsible for transferring a fatty acyl-group from the *sn*-2 position of a phospholipid (e.g., phosphatidylcholine) to the *sn*-3 position of 1,2-diacylglycerol to produce a lysophospholipid and TAG via an acyl-CoA-independent mechanism.

[0009] Furthermore, despite reports of a variety of conserved membrane bound O-acyltransferase ["MBOAT"] family protein motif sequences within LPCATs in both public and patent literature, a detailed investigation concerning specific mutations within these motifs has not been previously conducted.

SUMMARY OF THE INVENTION

[0010] In one embodiment, the invention concerns a recombinant *Yarrowia* cell for the production of at least one long-chain polyunsaturated fatty acid (PUFA) having a chain length of C20 or greater, wherein said recombinant cell has been genetically engineered to comprise a polyunsaturated fatty acid biosynthetic pathway capable of producing at least one said long-chain polyunsaturated fatty acid, and has been further modified to introduce:

1. (a) a chimeric gene encoding at least one polypeptide having acyl CoA:lysophosphatidylcholine acyltransferase (LPCAT) activity wherein said polypeptide has at least 90% amino acid identity, based on the Clustal W method of alignment, when compared to the amino acid sequence set forth in SEQ ID NO: 4 (YlLPCAT); and
2. (b) a chimeric gene encoding at least one polypeptide having phospholipid:diacylglycerol acyltransferase (PDAT) activity wherein said polypeptide has at least 90% amino acid identity, based on the Clustal W method of alignment, when compared to the amino acid sequence of SEQ ID NO: 32 (YlPDAT); and

wherein said chimeric genes each comprise a promoter which is heterologous to the coding sequence encoding the polypeptides of (a) and (b), and wherein the recombinant cell comprises an increased amount of a said PUFA measured as a weight percent of total fatty acids, when compared to a control cell which corresponds to the recombinant *Yarrowia* cell for production but which has not been modified to introduce the chimeric genes encoding the LPCAT or PDAT polypeptides of (a) or (b).

[0011] In a second embodiment, the recombinant *Yarrowia* cell further comprises at least one of the following, when compared to the control cell:

1. (i) an increased C_{18} to C_{20} elongation conversion efficiency, or
2. (ii) an increased amount of total fatty acids measured as a weight percent of dry cell weight.

Preferably, the increased C_{18} to C_{20} elongation conversion efficiency is an effect of increased delta-9 elongase conversion efficiency or increased delta-6 elongase conversion efficiency in the recombinant microbial cell. In a third embodiment, the polypeptide having PDAT activity has at least 95% amino acid identity, based on the Clustal W method of alignment, when compared to an amino acid sequence selected from the group consisting of SEQ ID NO:32 (YlPDAT), and/or wherein the polypeptide having acyl CoA:lysophosphatidylcholine acyltransferase activity has at least 95% amino acid identity, based on the Clustal W method of alignment, when compared to the amino acid sequence set forth in SEQ ID NO:4 (YlLPCAT).

[0012] In a fourth embodiment, the polypeptide having LPCAT activity is selected from the group consisting of:

1. (a) a polypeptide comprising at least one membrane-bound O-acyltransferase protein family motif selected from the group consisting of: SEQ ID NO:5 (WHG-X_3-GY-X_3-F), SEQ ID NO:6 (Y-X_4-F), SEQ ID NO:7 (Y-X_3-YF-X_2-H), SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG), SEQ ID NO:9 (RxKYY-X_2-W-X_3-[E/D]-[A/G]-X_5-GxG-[F/Y]-xG), SEQ ID NO:10 (EX_{11}WN-X_2-[T/V]-X_2-W), SEQ ID NO:11 (SAxWHG-X2-PGY-X2-[T/F]-F), SEQ ID NO:12 (M-[V/I]-[L/I/V]-[V/C/A/T]-[M/L/Q]-K-[L/V/I/M]-[S/T/Y/I]-[S/T/A/M/G]-[F/L/C/Y]-[C/A/G/S]-[W/Y/M/I/F/C]-[N/S/E/Q/D]-[V/Y/L/I]-[H/Y/A/N/S/T]-DG), SEQ ID NO:13 (R-[L/M/F/W/P/Y]-KYY-[G/A/F/H/S]-[V/A/I/C]-W-[Y/E/T/M/S/L]-[L/I/N]-[T/S/A]-[E/D]-[G/A]-[A/S/I/V]-[C/S/I/N/H/L]-[V/I/N]-[L/I/N/A/C]-[S/C/W/A/I]-G-[M/I/L/A/F]-G-[Y/F]-[N/E/S/T/R/K]-G), SEQ ID NO:14 (E-[T/F/L/M]-[A/S]-[Q/D/P/K/T]-[N/S]-[S/I/T/L/A/M/F]-[H/K/R/V]-[G/C/E/T/Q/D/M]-[Y/A/M/L/I/F]-[L/S/P/I]-[G/E/A/L/N/D]-[S/A/V/F/M/N]-WN-[K/M/I/C]-[N/K/Q/G]-[T/V]-[N/A/S]-[H/K/N/T/R/L]-W),

SEQ ID NO:15 (SA-[F/M/V/I]-WHG-[F/V/T/L]-[Y/S/R]-PGY-[Y/M/I]-[L/M/I/F]-[T/F]-F), SEQ ID NO:16 (M-[V/I]-L-X_2-KL), SEQ ID NO:17 (RxKYY-X_2-W), and SEQ ID NO:18 (SAxWHG); and

2. (b) a polypeptide comprising at least one mutant membrane-bound O-acyltransferase protein family motif selected from the group consisting of:
    1. (i) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:38, wherein SEQ ID NO:38 differs from SEQ ID NO:16 (M-[V/I]-L-X_2-KL) by at least one amino acid mutation selected from the group consisting of: V2C, I2C, L3A, L3C, L3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7I and L7M;
    2. (ii) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:39, wherein SEQ ID NO:39 differs from SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG) by at least one amino acid mutation selected from the group consisting of: V2C, I2C, L3A, L3C, L3G, I3A, I3C, I3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7M, V7A, V7N, V7G, V7H, V7M, I7A, I7N, I7G, I7H, I7M, D16Q, D16N, D16H, G17A, G17V and G17N;
    3. (iii) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:40 wherein SEQ ID NO:40 differs from SEQ ID NO:5 (WHG-X_3-GY-X_3-F) by at least one amino acid mutation selected from the group consisting of: F12N, F12C, F12G and F12T; and
    4. (iv)a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:41, wherein SEQ ID NO:41 differs from SEQ ID NO:11 (SAxWHG-X_2-PGY-X_2-[T/F]-F) by at least one amino acid mutation selected from the group consisting of: T14A, T14C, T14S, F14A, F14C, F14S, F15N, F15C, F15G and F15T;

[0013] In a fifth embodiment, the long-chain PUFA is selected from the group consisting of: eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, omega-6 docosapentaenoic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid, omega-3 docosapentaenoic acid and docosahexaenoic acid. Preferably, the PUFA is eicosapentaenoic acid.

[0014] The recombinant microbial cell may be selected from the group consisting of: algae, yeast, euglenoids, stramenopiles, oomycetes and fungi. Preferably, the recombinant microbial cell is an oleaginous yeast. The oleaginous yeast may be of the genus *Yarrowia*.

[0015] Another embodiment of the invention concerns a method for improving the production of at least one long-chain PUFA having a chain length of C20 or greater. This method comprises:

1. (a) growing the recombinant microbial cell of the invention in the presence of a fermentable carbon source; and
2. (b) optionally, recovering the long-chain PUFA.

[0016] In one aspect of the method, the recombinant microbial cell is an oleaginous yeast and the long-chain PUFA is selected from the group consisting of: eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, omega-6 docosapentaenoic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid, omega-3 docosapentaenoic acid and docosahexaenoic acid. Preferably, the PUFA is eicosapentaenoic acid. In another aspect of the method, the oleaginous yeast is of the genus *Yarrowia*.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

[0017]

FIG. 1 illustrates the cycle of phosphatidylcholine (PC) substrate use by PDAT and regeneration by LPCAT. PC_1 and PC_2 may differ in that the fatty acid removed from PC_1 by PDAT to yield lysophosphatidylcholine (LPC) may differ from the fatty acid added to LPC by LPCAT in yielding PC_2.

FIG. 2 illustrates the omega-3/omega-6 fatty acid biosynthetic pathway, and should be viewed together when considering the description of this pathway.

FIG. 3 provides plasmid maps for the following: (A) pY196 and (B) pY301.

FIG. 4 provides a plasmid map for pY306-N.

[0018] The invention can be more fully understood from the following detailed description and the accompanying sequence descriptions (Table 1), which form a part of this application.

Table 1. Summary of Gene and Protein SEQ ID NOs

Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
Saccharomyces cerevisiae Ale1 ("ScAle1" or "ScLPAAT"; also ORF "YOR175C")	1 (1860 bp)	2 (619 AA)
Yarrowia lipolytica Ale1 ("YlAle1" or "YlLPCAT") (YALI0F19514p)	3 (1539 bp)	4 (512 AA)
Shindou et al. WHG-X_3-GY-X_3-F motif	--	5
Shindou et al. Y-X_4-F motif	--	6
Shindou et al. Y-X_3-YF-X_2-H motif	--	7
U.S. Pat. Appl. Publ. No. 2008-0145867-A1 M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG motif	--	8
U.S. Pat. Appl. Publ. No. 2008-0145867-A1 RxKYY-X_2-W-X_3-[E/D]-[A/G]-X_5-GxG-[F/Y]-xG motif	--	9
U.S. Pat. Appl. Publ. No. 2008-0145867-A1 EX_{11}WN-X_2-[T/V]-X_2-W motif	--	10
U.S. Pat. Appl. Publ. No. 2008-0145867-A1 SAxWHG-X_2-PGY-X_2-[T/F]-F motif	--	11
U.S. Patent No. 7,732,155 motif	--	12
U.S. Patent No. 7,732,155 motif	--	13
U.S. Patent No. 7,732,155 motif	--	14
U.S. Patent No. 7,732,155 motif	--	15
U.S. Pat. Appl. Publ. No. 2010-0317882-A1 M-[V/I]-L-X_2-KL motif	--	16
U.S. Pat. Appl. Publ. No. 2010-0317882-A1 RxKYY-X_2-W motif	--	17
U.S. Pat. Appl. Publ. No. 2010-0317882-A1 SAxWHG motif	--	18
Mutant YlLPCAT, comprising a mutant Motif I motif and/or a mutant Motif II motif	--	19 (512 AA)
Mortierella alpina LPAAT1 ("MaLPAAT1")	20 (945 bp)	21 (314 AA)
Yarrowia lipolytica LPAAT1 ("YlLPAAT1")	22 (1549 bp)	23 (282 AA)
Saccharomyces cerevisiae LPAAT ("ScLPAAT"; also ORF "YDL052C")	--	24 (303 AA)
1-acyl-*sn*-glycerol-3-phosphate acyltransferase motif NHxxxxD	--	25
1-acyl-*sn*-glycerol-3-phosphate acyltransferase motif EGTR	--	26
Lewin et al. and Yamashita et al. 1-acyl-*sn*-glycerol-3-phosphate acyltransferase motif GxxFI-[D/R]-R	--	27
Yamashita et al. 1-acyl-*sn*-glycerol-3-phosphate acyltransferase motif [V/I]-[P/X]-[I/V/L]-[I/V]-P-[V/I]	--	28
Yamashita et al. 1-acyl-*sn*-glycerol-3-phosphate acyltransferase motif IVPIVM	--	29
Saccharomyces cerevisiae PDAT (GenBank Accession No. P40345)	--	30 (661 AA)
Yarrowia lipolytica phospholipid:diacylglycerol acyltransferase ("YlPDAT")	31 (1947 bp)	32 (648 AA)
Mutant M-[V/I]-L-X_2-KL motif	--	33
Mutant M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG motif	--	34
Mutant WHG-X_3-GY-X_3-F motif	--	35
Mutant SAxWHG-X_2-PGY-X_2-[T/F]-F motif	--	36
Mutant YlLPCAT, comprising single mutations in Motif I and/or Motif II	--	37 (512 AA)
Mutant M-[V/I]-L-X_2-KL motif	--	38
Mutant M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG motif	--	39
Mutant WHG-X_3-GY-X_3-F motif	--	40
Mutant SAxWHG-X_2-PGY-X_2-[T/F]-F motif	--	41
Mutant YlLPCAT, comprising a single mutation in Motif I and a single mutation in Motif II	--	42 (512 AA)

Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
Plasmid pY196 for co-expressing PDAT and LPAAT	43 (11017 bp)	
Plasmid pY301 for co-expressing PDAT and LPCAT	44 (10575 bp)	
"YILPCAT*", YILPCAT lacking two internal *Nco*I restriction sites with respect to SEQ ID NO:3, but encoding wild type YILPCAT protein	45 (1549 bp)	46 (512 AA)
Plasmid pY306, containing YILPCAT	47 (8518 bp)	
Plasmid pY306-N, containing YILPCAT*	48 (8518 bp)	
YILPCAT_M132X, comprising M132A, M132N, M132C, M132G, M132Q, M132H, M132I, M132L, M132F, M132P, M132S, M132T, M132W, M132Y or M132V mutation in Motif I	--	49
YILPCAT_V133X, comprising V133A, V133N, V133C, V133G, V133Q, V133H, V133L, V133M, V133F, V133P, V133S, V133T, V133W or V133Y mutation in Motif I	--	50
YILPCAT_L134X, comprising L134A, L134N, L134C, L134G, L134Q, L134H, L134M, L134F, L134P, L134S, L134T, L134W, L134Y or L134V mutation in Motif I	--	51
YILPCAT_C135X, comprising C135R, C135N, C135D, C135G, C135E, C135Q, C135H, C135I, C135L, C135K, C135M, C135F, C135P, C135S, C135W or C135Y mutation in Motif I	--	52
YILPCAT_M136X, comprising M136A, M136N, M136C, M136G, M136H, M136I, M136F, M136P, M136S, M136T, M136W, M136Y or M136V mutation in Motif I	--	53
YILPCAT_K137X, comprising K137A, K137R, K137N, K137G, K137H, K137P, K137S, K137T, or K137Y mutation in Motif I	--	54
YILPCAT_L138X, comprising L138A, L138N, L138C, L138G, L138Q, L138H, L138I, L138M, L138F, L138P, L138S, L138T, L138W, or L138Y mutation in Motif I	--	55
YILPCAT_S139X, comprising S139A, S139N, S139C, S139G, S139H, S139L, S139M, S139F, S139P, S139W, or S139V mutation in Motif I	--	56
YILPCAT_S140X, comprising S140N, S140C, S140H, S1401, S140L, S140F, S140P, S140W, S140Y or S140V mutation in Motif I	--	57
YILPCAT_F141X, comprising F141A, F141N, F141G, F141H, F141I, F141M, F141P, F141S,	--	58
F141T, F141W, or F141V mutation in Motif I		
YILPCAT_G142X, comprising G142N, G142H, G142I, G142L, G142M, G142F, G142P, G142T, G142W, G142Y or G142V mutation in Motif I	--	59
YILPCAT_W143X, comprising W143A, W143G, W143H, W143L, W143K, W143P, W143S, W143T or W143V mutation in Motif I	--	60
YILPCAT_N144X, comprising N144A, N144R, N144G, N144H, N144K, N144F, N144P, N144T or N144V mutation in Motif I	--	61
YILPCAT_V145X, comprising V145A, V145C, V145G, V145E, V145H, V145M, V145F, V145P, V145S, V145T, or V145W mutation in Motif I	--	62
YILPCAT_Y146X, comprising Y146R, Y146N, Y146D, Y146G, Y146E, Y146Q, Y146I, Y146L, Y146M, Y146F, Y146P, Y146W or Y146V mutation in Motif I	--	63
YILPCAT_D147X, comprising D147A, D147N, D147G, D147E, D147Q, D147H, D147F, D147S, or D147T mutation in Motif I	--	64
YILPCAT_G148X, comprising G148A, G148N, G148H, G148L, G148M, G148F, G148S, G148T or G148V mutation in Motif I	--	65
YILPCAT_S376X, comprising S376A, S376G, S376H, S376L, S376F, S376P, S376T or S376V mutation in Motif II	--	66
YILPCAT_A377X, comprising A377N, A377G, A377H, A377L, A377F, A377P, A377S, A377T or A377V mutation in Motif II	--	67
YILPCAT_F378X, comprising F378A, F378N, F378C, F378G, F378H, F378L, F378P, F378S, F378T, F378W, or F378Y mutation in Motif II	--	68
YILPCAT_T382X, comprising T382A, T382N, T382G, T382Q, T382H, T382I, T382M, T382P, T382S, T382W and or T382Y mutation in Motif II	--	69
YILPCAT_R383X, comprising R383A, R383N, R383D, R383G, R383E, R383Q, R383H, R383I, R383L, R383K, R383M, R383F, R383P, R383T, R383W or R383V mutation in Motif II	--	70
YILPCAT_P384X, comprising P384A, P384R, P384G, P384H, P384I, P384L, P384K,	--	71

Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
P384M, P384F, P384S, P384T, P384W, P384Y or P384V mutation in Motif II		
YILPCAT_G385X, comprising G385A, G385N, G385C, G385G, G385H, G385I, G385L, G385K, G385M, G385F, G385S, G385T, G385W, G385Y or G385V mutation in Motif II	--	72
YILPCAT_Y386X, comprising Y386A, Y386G, Y386H, Y386L, Y386F, Y386P, Y386S, Y386T or	--	73
Y386V mutation in Motif II		
YILPCAT_Y387X, comprising Y387A, Y387G, Y387H, Y387L, Y387F, Y387P, Y387S, Y387T, Y387W or Y387V mutation in Motif II	--	74
YILPCAT_L388X, comprising L388A, L388G, L388H, L388P, L388S, L388T, L388W, L388Y or L388V mutation in Motif II	--	75
YILPCAT_T389X, comprising T389A, T389C, T389G, T389H, T389I, T389L, T389M, T389F, T389P, T389S, T389W, T389Y or T389V mutation in Motif II	--	76
YILPCAT_F390X, comprising F390A, F390N, F390C, F390G, F390H, F390L, F390M, F390P, F390S, F390T or F390V mutation in Motif II	--	77
YILPCAT comprising M136S_T389A	78	79
YILPCAT comprising M136S_T389C	80	81
YILPCAT comprising M136S_T389S	82	83
YILPCAT comprising M136V_T389C	84	85
YILPCAT comprising N144A_F390S	86	87
YILPCAT comprising G148A_F390S	88	89
YILPCAT comprising G148N_T382I	90	91
YILPCAT comprising G148N_F390S	92	93

DETAILED DESCRIPTION OF THE INVENTION

[0019] When an amount, concentration, or other value or parameter is given as either a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the invention be limited to the specific values recited when defining a range.

[0020] As used herein the term "invention" or "present invention" is intended to refer to all aspects and embodiments of the invention as described in the claims and specification herein and should not be read so as to be limited to any particular embodiment or aspect.

[0021] In this disclosure, a number of terms and abbreviations are used. Amino acids are identified by either the one-letter code or the three-letter codes for amino acids, in conformity with the IUPAC-IYUB standards described in Nucleic Acids Research, 13:3021-3030 (1985) and in the Biochemical Journal, 219 (2):345-373 (1984).

[0022] "Open reading frame" is abbreviated as "ORF".

[0023] "Polymerase chain reaction" is abbreviated as "PCR".

[0024] "American Type Culture Collection" is abbreviated as "ATCC".

[0025] "Polyunsaturated fatty acid(s)" is abbreviated as "PUFA(s)".

[0026] "Long-chain polyunsaturated fatty acid(s)" is abbreviated as "LC-PUFA(s)".

[0027] "Triacylglycerols" are abbreviated as "TAGs".

[0028] "Total fatty acids" are abbreviated as "TFAs".

[0029] "Fatty acid methyl esters" are abbreviated as "FAMEs".

[0030] "Dry cell weight" is abbreviated as "DCW".

[0031] "Acyl-CoA:lysophospholipid acyltransferase(s)" or "lysophospholipid acyltransferase(s)" is abbreviated as "LPLAT(s)".

[0032] "Lysophosphatidylcholine acyltransferase(s)" is abbreviated as "LPCAT(s)".

[0033] "Membrane bound *O*-acyltransferase" is abbreviated as "MBOAT".

[0034] "Phospholipid:diacylglycerol acyltransferase(s)" is abbreviated as "PDAT(s)".

[0035] The term "glycerophospholipids" refers to a broad class of molecules, having a glycerol core with fatty acids at the *sn-1* position and *sn*-2 position, and a polar head group (e.g., phosphate, choline, ethanolamine, glycerol, inositol, serine, cardiolipin) joined at the *sn*-3 position via a phosphodiester bond. Glycerophospholipids thus include phosphatidylcholine ["PC"], phosphatidylethanolamine ["PE"], phosphatidylglycerol ["PG"], phosphatidylinositol ["PI"], phosphatidylserine ["PS"] and cardiolipin ["CL"].

[0036] "Lysophospholipids" are derived from glycerophospholipids by deacylation of the *sn*-2 position. Lysophospholipids include, e.g., lysophosphatidic acid ["LPA"], lysophosphatidylcholine ["LPC"], lysophosphatidylethanolamine ["LPE"], lysophosphatidylserine ["LPS"], lysophosphatidylglycerol ["LPG"] and lysophosphatidylinositol ["LPI"].

[0037] The term "acyltransferase" refers to an enzyme responsible for transferring an acyl group from a donor lipid to an acceptor lipid molecule.

[0038] The term "acyl-CoA:lysophospholipid acyltransferase" or "lysophospholipid acyltransferase" ["LPLAT"] refers to a broad class of acyltransferases having the ability to acylate a variety of lysophospholipid substrates at the *sn*-2 position. A variety of LPLATs have been identified, including LPAATs (catalyzing conversion of LPA to PA), LPEATs (catalyzing conversion of LPE to PE), LPSATs (catalyzing conversion of LPS to PS), LPGATs (catalyzing conversion of LPG to PG), and LPIATs (catalyzing conversion of LPI to PI). LPC acyltransferases ["LPCATs"] are the focus of the present application, having the ability to catalyze conversion of LPC to PC. Standardization of LPLAT nomenclature has not been formalized, so various other designations are used in the art (for example, LPCATs are often referred to as acyl-CoA:1-acyl lysophosphatidyl-choline acyltransferases). Additionally, it is important to note that some LPLATs, such as the *Saccharomyces cerevisiae* Ale1 (ORF YOR175C, SEQ ID NO:2), have broad specificity and thus a single enzyme may be capable of catalyzing several LPLAT reactions, including LPAAT, LPCAT and LPEAT reactions (Tamaki et al., J. Biol. Chem., 282:34288-34298 (2007); Ståhl et al., FEBS Letters, 582:305-309 (2008); Chen et al., FEBS Letters, 581:5511-5516 (2007); Benghezal et al., J. Biol. Chem., 282:30845-30855 (2007); Riekhof et al., J. Biol. Chem., 282:28344-28352 (2007)).

[0039] More specifically, the term "polypeptide having lysophosphatidylcholine acyltransferase ["LPCAT"] activity" will refer to those enzymes capable of catalyzing the reaction: acyl-CoA + 1-acyl-*sn*-glycero-3-phosphocholine → CoA + 1,2-diacyl-*sn*-glycero-3-phosphocholine (EC 2.3.1.23). LPCAT activity has been described in two structurally distinct protein families, i.e., the LPAAT protein family (Hishikawa et al., Proc. Natl. Acad. Sci. U.S.A., 105:2830-2835 (2008); Intl. Appl. Publ. No. WO 2004/076617) and the ALE1 protein family (Tamaki et al., Ståhl et al., Chen et al., Benghezal et al., Riekhof et al.).

[0040] The term "LPCAT" refers to a protein of the ALE1 protein family that: 1) has LPCAT activity (EC 2.3.1.23) and shares at least about 45% amino acid identity, based on the Clustal W method of alignment, when compared to an amino acid sequence selected from the group consisting of SEQ ID NO:2 (ScAle1) and SEQ ID NO:4 (YlAle1); and/or 2) has LPCAT activity (EC 2.3.1.23) and has at least one membrane bound O-acyltransferase ["MBOAT"] protein family motif, described below. Examples of ALE1 polypeptides include ScAle1 and YlLPCAT.

[0041] The term "ScAle1" refers to an LPCAT isolated from *Saccharomyces cerevisiae* (ORF "YOR175C"). ScAle1 may have the amino acid sequence of SEQ ID NO:2, encoded by the nucleotide sequence set forth as SEQ ID NO:1.

[0042] The term "YlAle1" or "YlLPCAT" refers to a LPCAT isolated from *Yarrowia lipolytica.* YlLPCAT may have the amino acid sequence of SEQ ID NO:4, encoded by the nucleotide sequence set forth as SEQ ID NO:3.

[0043] The term "conserved domain" or "motif" means a set of amino acids conserved at specific positions along an aligned sequence of evolutionarily related proteins. While amino acids at other positions can vary between homologous proteins, amino acids that are highly conserved at specific positions likely indicate amino acids that are essential in the structure, the stability, or the activity of a protein. Because they are identified by their high degree of conservation in aligned sequences of a family of protein

homologues, they can be used as identifiers, or "signatures", to determine if a protein with a newly determined sequence belongs to a previously identified protein family.

[0044] A variety of membrane bound O-acyltransferase ["MBOAT"] family motifs have been proposed. These motifs are summarized in Table 2 below and discussed further in U.S. Pat. Appl. Publ. No. 2010-0317882-A1.

Table 2. Membrane Bound *O*-Acyltransferase ["MBOAT"] Family Motifs

Reference	Organisms Analyzed	Motif (X represents any amino acid)	SEQ ID NO
Shindou et al. (Biochem. Biophys. Res. Comm., 383:320-325 (2009))	*Homo sapiens, Gallus gallus, Danio rerio Caenorhabditis elegans*	WD	-
		WHG-X_3-GY-X_3-F	5
		Y-X_4-F	6
		Y-X_3-YF-X_2-H	7
U.S. Pat. Appl. Publ. No. 2008-0145867-A1	Non-plants	M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG	8
		RxKYY-X_2-W-X_3-[E/D]-[A/G]-X_5-GxG-[F/Y]-xG	9
		EX_{11}WN-X_2-[T/V]-X_2-W	10
		SAxWHG-X_2-PGY-X_2-[T/F]-F	11
U.S. Pat. No. 7,732,155	Non-plants	M-[V/I]-[L/I/V]-[V/C/A/T]-[M/L/Q]-K-[L/V/I/M]-[S/T/Y/I]-[S/T/A/M/G]-[F/L/C/Y]-[C/A/G/S]-[W/Y/M/I/F/C]-[N/S/E/Q/D]-[V/Y/L/I]-[H/Y/A/N/S/T]-DG	12
		R-[L/M/F/W/P/Y]-KYY-[G/A/F/H/S]-[V/A/I/C]-W-[Y/E/T/M/S/L]-[L/I/N]-[T/S/A]-[E/D]-[G/A]-[A/S/I/V]-[C/S/I/N/H/L]-[V/I/N]-[L/I/N/A/C]-[S/C/W/A/I]-G-[M/I/L/A/F]-G-[Y/F]-[N/E/S/T/R/K]-G	13
		E-[T/F/L/M]-[A/S]-[Q/D/P/K/T]-[N/S]-[S/I/T/L/A/M/F]-[H/K/R/V]-[G/C/E/T/Q/D/M]-[Y/A/M/L/I/F]-[L/S/P/I]-[G/E/A/L/N/D]-[S/A/V/F/M/N]-WN-[K/M/I/C]-[N/K/Q/G]-[T/V]-[N/A/S]-[H/K/N/T/R/L]-W	14
		SA-[F/M/V/I]-WHG-[F/V/T/L]-[Y/S/R]-PGY-[Y/M/I]-[L/M/I/F]-[T/F]-F	15
U.S. Pat. Appl. Publ. No. 2010-0317882-A1	Yeast and Fungi	M-[V/I]-L-X_2-KL	16
		RxKYY-X_2-W	17
		E-X_{11}-WN-X_2-[T/V]-X_2-W	10
		SAxWHG	18

[0045] The term "mutant polypeptide having LPCAT activity comprising at least one mutant membrane bound *O*-acyltransferase ["MBOAT"] protein family motif" or "mutant polypeptide having LPCAT activity comprising at least one mutant MBOAT motif" refers to a polypeptide of the present invention comprising at least one amino acid mutation with respect to SEQ ID NOs:5-18.

[0046] For each amino acid substitution in an MBOAT motif disclosed herein, the first letter corresponds to the amino acid in the wild type MBOAT motif and the second letter corresponds to the amino acid found in the same position in the mutant MBOAT motif, e.g., an L3A mutation in SEQ ID NO:16 [M-[V/I]-L-X_2-KL] indicates a change from Leu [L] in SEQ ID NO:16 at position 3 to Ala [A] in the MBOAT mutant. This nomenclature is used throughout the specification to refer to mutations within the LPCAT motifs and proteins described herein; similar notation is used to describe substitutions within nucleotide sequences (e.g., A9G indicates a change from adenine [A] at base position 9 in the nucleotide sequence encoding an MBOAT motif to guanine [G]).

[0047] Preferably, a mutant polypeptide having at least LPCAT activity comprising at least one mutant MBOAT motif (e.g., a mutated form of one of SEQ ID NOs:5-8) will have equivalent or improved LPCAT activity when compared to a control polypeptide

having LPCAT activity comprising at least one MBOAT motif (e.g., one of SEQ ID NOs:5-18) that is the wild type version of the mutated MBOAT motif in the mutant polypeptide.

[0048] Although "mutations" may include any deletions, insertions and point mutations (or combinations thereof), in a preferred embodiment, a mutant LPCAT having lysophosphatidylcholine acyltransferase ["LPCAT"] activity comprising at least one mutant MBOAT motif is set forth in SEQ ID NO:19, wherein SEQ ID NO:19 differs from SEQ ID NO:4 [YILPCAT] by at least one amino acid mutation, wherein:

1. (a) one of the at least one amino acid mutations is in an amino acid residue selected from the group consisting of: residue 133, residue 134, residue 135, residue 136, residue 137, residue 138, residue 139, residue 140, residue 141, residue 142, residue 143, residue 144, residue 145, residue 146, residue 147, residue 148;
2. (b) one of the at least one amino acid mutations is in an amino acid residue selected from the group consisting of: residue 378, residue 382, residue 383, residue 385, residue 388, residue 389 and residue 390; and/or
3. (c) said at least one amino acid mutation comprises at least two amino acid mutations, wherein:
    1. (i) the first amino acid mutation is in an amino acid residue selected from the group set forth in part (a), and
    2. (ii) the second amino acid mutation is in an amino acid residue selected from the group set forth in part (b).

[0049] The term "LPCAT" also refers to a protein that has LPCAT activity (EC 2.3.1.23) and which may also have an alternate acyl-CoA:lysophospholipid acyltransferase activity (e.g., LPAAT activity, LPEAT activity, LPSAT activity, LPGAT activity, LPIAT activity). For example, a polypeptide may have both LPCAT and LPAAT activity and should thus be considered as an LPCAT herein, despite being classified in previous literature as an LPAAT polypeptide. These LPCATs may possess structural characteristics of LPAAT proteins.

[0050] The term "polypeptide having lysophosphatidic acid acyltransferase ["LPAAT"] activity" will refer to those enzymes capable of catalyzing the reaction: acyl-CoA + 1-acyl-*sn*-glycerol 3-phosphate → CoA + 1,2-diacyl-*sn*-glycerol 3-phosphate (EC 2.3.1.51).

[0051] The term "LPAAT" refers to a protein that: 1) has LPAAT activity and shares at least about 43.9% amino acid identity, based on the Clustal W method of alignment, when compared to an amino acid sequence selected from the group consisting of SEQ ID NO:21 (MaLPAAT1), SEQ ID NO:23 (YILPAAT1) and SEQ ID NO:24 (ScLPAAT1); and/or 2) has LPAAT activity and has at least one 1-acyl-*sn*-glycerol-3-phosphate acyltransferase family motif selected from the group consisting of: NHxxxxD (SEQ ID NO:25) and EGTR (SEQ ID NO:26). More specifically, Lewin et al. (Biochemistry, 38:5764-5771 (1999)) and Yamashita et al. (Biochim, Biophys. Acta, 1771:1202-1215 (2007)) proposed the following 1-acyl-sn-glycerol-3-phosphate acyltransferase ["LPAAT"] family motifs to be important for "acyl-CoA:lysophospholipid acyltransferase" or "lysophospholipid acyltransferase" ["LPLAT"] activity, based on alignment of sequences from bacteria, yeast, nematodes and mammals: NHxxxxD (SEQ ID NO:25), GxxFI-[D/R]-R (SEQ ID NO:27), EGTR (SEQ ID NO:26) and either [V/I]-[P/X]-[I/V/L]-[I/V]-P-[V/I] (SEQ ID NO:28) or IVPIVM (SEQ ID NO:29). Examples of LPAAT polypeptides include ScLPAAT, MaLPAAT1 and YILPAAT1.

[0052] The term "ScLPAAT" refers to an LPAAT isolated from *Saccharomyces cerevisiae* (e.g., ORF "YDL052C", SEQ ID NO:24).

[0053] The term "MaLPAAT1" refers to an LPAAT isolated from *Mortierella alpina.* MaLPAAT1 may have the amino acid sequence of SEQ ID NO:21, encoded by the nucleotide sequence set forth as SEQ ID NO:20. The NHxxxxD (SEQ ID NO:25) and EGTR (SEQ ID NO:26) motifs are present in MaLPAAT1, but the other LPAAT motifs are not.

[0054] The terms "YILPAAT1" and "YILPAAT2" refer to LPAATs isolated from *Yarrowia lipolytica.* An YILPAAT may have the amino acid sequence of SEQ ID NO:23, encoded by the nucleotide sequence set forth as SEQ ID NO:22. The NHxxxxD (SEQ ID NO:25) and EGTR (SEQ ID NO:26) motifs are present in YILPAAT1, but the other LPAAT motifs are not.

[0055] The term "polypeptide having phospholipid:diacylglycerol acyltransferase ["PDAT"] activity" will refer to those enzymes capable of transferring a fatty acyl group from the *sn-2* position of a phospholipid (e.g., phosphatidylcholine) to the *sn*-3 position of 1,2-diacylglycerol [E.C.2.3.1.158], thus resulting in a lysophospholipid and TAG. Although both PDATs and acyl-CoA:diacylglycerol acyltransferases (DGATs) [E.C. 2.3.1.20] are involved in the terminal step of TAG biosynthesis, only PDAT may synthesize TAGs via an acyl-CoA-independent mechanism. A representative PDAT enzyme, as set forth in SEQ ID NO:30, is encoded by the LRO1 gene in *Saccharomyces cerevisiae* (Dahlqvist et al., Proc. Natl. Acad. Sci. U.S.A., 97:6487 (2000)).

[0056] The term "YIPDAT" refers to a PDAT isolated from *Yarrowia lipolytica.* YIPDAT may have the amino acid sequence of SEQ ID NO:32, encoded by the nucleotide sequence set forth as SEQ ID NO:31 (U.S. Pat. 7,901,928).

[0057] The term "ortholog" refers to a homologous protein from a different species that evolved from a common ancestor protein as evidenced by being in one clade of a phylogenetic tree analysis and that catalyzes the same enzymatic reaction.

[0058] The term "oil" refers to a lipid substance that is liquid at 25 °C and usually polyunsaturated. In oleaginous organisms, oil

constitutes a major part of the total lipid. "Oil" is composed primarily of triacylglycerols ["TAGs"] but may also contain other neutral lipids, phospholipids and free fatty acids. The fatty acid composition in the oil and the fatty acid composition of the total lipid are generally similar; thus, an increase or decrease in the concentration of PUFAs in the total lipid will correspond with an increase or decrease in the concentration of PUFAs in the oil, and vice versa.

[0059] "Neutral lipids" refer to those lipids commonly found in cells in lipid bodies as storage fats and are so called because at cellular pH, the lipids bear no charged groups. Generally, they are completely non-polar with no affinity for water. Neutral lipids generally refer to mono-, di-, and/or triesters of glycerol with fatty acids, also called monoacylglycerol, diacylglycerol or triacylglycerol, respectively, or collectively, acylglycerols. A hydrolysis reaction must occur to release free fatty acids from acylglycerols.

[0060] The term "triacylglycerols" ["TAGs"] refers to neutral lipids composed of three fatty acyl residues esterified to a glycerol molecule. TAGs can contain LC-PUFAs and saturated fatty acids, as well as shorter chain saturated and unsaturated fatty acids.

[0061] The term "total fatty acids" ["TFAs"] herein refers to the sum of all cellular fatty acids that can be derivatized to fatty acid methyl esters ["FAMEs"] by the base transesterification method (as known in the art) in a given sample, which may be the biomass or oil, for example. Thus, total fatty acids include fatty acids from neutral lipid fractions (including diacylglycerols, monoacylglycerols and TAGs) and from polar lipid fractions (including the PC and the PE fractions), but not free fatty acids.

[0062] The term "total lipid content" of cells is a measure of TFAs as a percent of the dry cell weight ["DCW"], although total lipid content can be approximated as a measure of FAMEs as a percent of the DCW ["FAMEs % DCW"]. Thus, total lipid content ["TFAs % DCW"] is equivalent to, e.g., milligrams of total fatty acids per 100 milligrams of DCW. The total lipid content can also refer to the oil content.

[0063] The concentration of a fatty acid in the total lipid is expressed herein as a weight percent of TFAs ["% TFAs"], e.g., milligrams of the given fatty acid per 100 milligrams of TFAs. Unless otherwise specifically stated herein, reference to the percent of a given fatty acid with respect to total lipids is equivalent to concentration of the fatty acid as % TFAs (e.g., % EPA of total lipids is equivalent to EPA % TFAs).

[0064] In some cases, it is useful to express the content of a given fatty acid(s) in a cell as its weight percent of the dry cell weight ["% DCW"]. Thus, for example, EPA % DCW would be determined according to the following formula: (EPA % TFAs) * (TFAs % DCW)]/100. The content of a given fatty acid(s) in a cell as its weight percent of the dry cell weight ["% DCW"] can be approximated, however, as: (EPA % TFAs) * (FAMEs % DCW)]/100.

[0065] The terms "lipid profile" and "lipid composition" are interchangeable and refer to the amount of individual fatty acids contained in a particular lipid fraction, such as in the total lipids or the oil, wherein the amount is expressed as a weight percent of TFAs. The sum of each individual fatty acid present in the mixture should be 100.

[0066] The term "fatty acids" refers to long chain aliphatic acids (alkanoic acids) of varying chain lengths, from about C_{12} to C_{22}, although both longer and shorter chain-length acids are known. The predominant chain lengths are between C_{16} and C_{22}. The structure of a fatty acid is represented by a simple notation system of "X:Y", where X is the total number of carbon ["C"] atoms in the particular fatty acid and Y is the number of double bonds. Additional details concerning the differentiation between "saturated fatty acids" versus "unsaturated fatty acids", "monounsaturated fatty acids" versus "polyunsaturated fatty acids" ["PUFAs"], and "omega-6 fatty acids" ["*n*-6"] versus "omega-3 fatty acids" ["*n*-3"] are provided in U.S. Patent 7,238,482.

[0067] Nomenclature used to describe PUFAs herein is given in Table 3. In the column titled "Shorthand Notation", the omega-reference system is used to indicate the number of carbons, the number of double bonds and the position of the double bond closest to the omega carbon, counting from the omega carbon, which is numbered 1 for this purpose. The remainder of Table 3 summarizes the common names of omega-3 and omega-6 fatty acids and their precursors, the abbreviations that will be used throughout the specification and the chemical name of each compound.

Table 3. Nomenclature of Polyunsaturated Fatty Acids and Precursors

Common Name	Abbreviation	Chemical Name	Shorthand Notation
Myristic	--	tetradecanoic	14:0
Palmitic	Palmitate	hexadecanoic	16:0
Palmitoleic	--	9-hexadecenoic	16:1
Stearic	--	octadecanoic	18:0
Oleic	--	*cis*-9-octadecenoic	18:1
Linoleic	LA	*cis*-9,12-octadecadienoic	18:2 omega-6
Gamma-Linolenic	GLA	*cis*-6, 9, 12-octadecatrienoic	18:3 omega-6

Common Name	Abbreviation	Chemical Name	Shorthand Notation
Eicosadienoic	EDA	*cis*-11, 14-eicosadienoic	20:2 omega-6
Dihomo-Gamma-Linolenic	DGLA	*cis*-8, 11, 14-eicosatrienoic	20:3 omega-6
Arachidonic	ARA	*cis*-5, 8, 11, 14-eicosatetraenoic	20:4 omega-6
Alpha-Linolenic	ALA	*cis*-9, 12, 15-octadecatrienoic	18:3 omega-3
Stearidonic	STA	*cis*-6, 9, 12, 15-octadecatetraenoic	18:4 omega-3
Eicosatrienoic	ETrA	*cis*-11, 14, 17-eicosatrienoic	20:3 omega-3
Eicosatetraenoic	ETA	*cis*-8, 11, 14, 17-eicosatetraenoic	20:4 omega-3
Eicosapentaenoic	EPA	*cis*-5, 8, 11, 14, 17-eicosapentaenoic	20:5 omega-3
Docosa-tetraenoic	DTA	*cis*-7, 10, 13, 16-docosatetraenoic	22:4 omega-6
Docosapentaenoic	DPAn-6	*cis*-4, 7, 10, 13, 16-docosapentaenoic	22:5 omega-6
Docosapentaenoic	DPA	*cis*-7, 10, 13, 16, 19-docosapentaenoic	22:5 omega-3
Docosahexaenoic	DHA	*cis*-4, 7, 10, 13, 16, 19-docosahexaenoic	22:6 omega-3

Although the omega-3/omega-6 PUFAs listed in Table 3 are the most likely to be accumulated in the oil fractions of microbial and plant hosts using the methods described herein, this list should not be construed as limiting or as complete.

[0068] The term "long-chain polyunsaturated fatty acid" ["LC-PUFA"] refers to those PUFAs that have chain lengths of C_{20} or greater. Thus, the term LC-PUFA includes at least EDA, DGLA, ARA, ETrA, ETA, EPA, DTA, DPAn-6, DPA and DHA.

[0069] The term "PUFA biosynthetic pathway" refers to a metabolic process that converts oleic acid to omega-6 fatty acids such as LA, EDA, GLA, DGLA, ARA, DTA and DPAn-6 and omega-3 fatty acids such as ALA, STA, ETrA, ETA, EPA, DPA and DHA. This process is well described in the literature (e.g., see U.S. Patent 7,7932,077). Briefly, this process involves elongation of the carbon chain through the addition of carbon atoms and desaturation of the molecule through the addition of double bonds, via a series of special elongation and desaturation enzymes termed "PUFA biosynthetic pathway enzymes" that are present in the endoplasmic reticulum membrane. More specifically, "PUFA biosynthetic pathway enzymes" refer to any of the following enzymes (and genes which encode said enzymes) associated with the biosynthesis of a PUFA, including: delta-4 desaturases, delta-5 desaturases, delta-6 desaturases, delta-12 desaturases, delta-15 desaturases, delta-17 desaturases, delta-9 desaturases, delta-8 desaturases, delta-9 elongases, $C_{14/16}$ elongases, $C_{16/18}$ elongases, $C_{18/20}$ elongases and/or $C_{20/22}$ elongases.

[0070] The term "PUFA biosynthetic pathway capable of producing at least one long-chain polyunsaturated product fatty acid" refers to a PUFA biosynthetic pathway comprising PUFA biosynthetic pathway enzymes that enables production of at least one long-chain polyunsaturated product fatty acid. FIG 2. depicts examples of PUFA biosynthetic pathways.

[0071] The terms "conversion efficiency" and "percent substrate conversion" refer to the efficiency by which a particular enzyme, such as a desaturase or elongase, can convert substrate to product. The conversion efficiency is measured according to the following formula: ([product]/[substrate+product]) *100, where 'product' includes the immediate product and all products derived from it. More specifically, since each PUFA biosynthetic pathway enzyme rarely functions with 100% efficiency to convert substrate to product, the final lipid profile of unpurified oils produced in a host cell will typically be a mixture of various PUFAs consisting of the desired omega-3/omega-6 fatty acid, as well as various upstream intermediary PUFAs. Thus, each enzyme's conversion efficiency is often considered, when optimizing biosynthesis of a desired fatty acid in a specific host organism.

[0072] The term "C_{18} to C_{20} elongation conversion efficiency" refers to the efficiency by which $C_{18/20}$ elongases can convert C_{18} substrates (i.e., LA, ALA, GLA, STA, etc.) to C_{20} products (i.e., EDA, ETrA, DGLA, ETA, EPA, etc.). These $C_{18/20}$ elongases can be either delta-9 elongases or delta-6 elongases.

[0073] The terms "delta-9 elongation conversion efficiency" and "delta-9 elongase conversion efficiency" refer to the efficiency by which delta-9 elongase can convert C_{18} substrates (i.e., LA, ALA) to C_{20} products (such as EDA, ETrA, DGLA, ETA, ARA, EPA). Delta-9 elongase conversion efficiency is referred to herein as "% Conv." or "d9e CE(%)".

[0074] The terms "delta-6 elongation conversion efficiency" and "delta-6 elongase conversion efficiency" refer to the efficiency by which delta-6 elongase can convert C_{18} substrates (such as GLA, STA) to C_{20} products (such as DGLA, ETA, ARA, EPA, etc.).

[0075] The term "increased" herein means having a greater quantity or activity, for example a quantity or activity only slightly greater than the original quantity or activity, or for example a quantity or activity in large excess compared to the original quantity or activity, and including all quantities or activities in between. Alternatively, "increased" may refer to a quantity or activity that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11 %, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% more than the quantity or activity for which the increased quantity or activity is being compared.

[0076] The terms "microbial cell" and "microbial organism" are used interchangeably herein and refer to a microorganism capable of receiving foreign or heterologous genes and capable of expressing those genes. A "recombinant microbial cell" refers to a microbial host cell that has been recombinantly engineered.

[0077] Generally, the term "oleaginous" refers to those organisms that tend to store their energy source in the form of oil (Weete, In: Fungal Lipid Biochemistry, 2nd Ed., Plenum, 1980). For the purposes of the present application, the term "oleaginous" refers to those microorganisms that can accumulate at least about 25% of their dry cell weight ["DCW"] as oil.

[0078] The term "oleaginous yeast" refers to those oleaginous microorganisms classified as yeasts that can make oil, i.e., wherein the oil can accumulate in excess of about 25% of their DCW. Examples of oleaginous yeast include the following genera: *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon* and *Lipomyces.* The ability to accumulate oil in excess of about 25% of the DCW of the yeast may be through efforts of recombinant engineering or through the natural abilities of the organism.

[0079] The term "conservative amino acid substitution" refers to a substitution of an amino acid residue in a given protein with another amino acid, without altering the chemical or functional nature of that protein. For example, it is well known in the art that alterations in a gene that result in the production of a chemically equivalent amino acid at a given site (but do not affect the structural and functional properties of the encoded, folded protein) are common. For the purposes herein, "conservative amino acid substitutions" are defined as exchanges within one of the following five groups:

1. 1. Small aliphatic, nonpolar or slightly polar residues: Ala [A], Ser [S], Thr [T] (Pro [P], Gly [G]);
2. 2. Polar, negatively charged residues and their amides: Asp [D], Asn [N], Glu [E], Gin [Q];
3. 3. Polar, positively charged residues: His [H], Arg [R], Lys [K];
4. 4. Large aliphatic, nonpolar residues: Met [M], Leu [L], Ile [I], Val [V] (Cys [C]); and
5. 5. Large aromatic residues: Phe [F], Tyr [Y], Trp [W].

Thus, Ala, a slightly hydrophobic amino acid, may be substituted by another less hydrophobic residue (e.g., Gly). Similarly, changes which result in substitution of one negatively charged residue for another (e.g., Asp for Glu) or one positively charged residue for another (e.g., Lys for Arg) can also be used to produce a functionally equivalent product. As such, conservative amino acid substitutions generally maintain: 1) the structure of the polypeptide backbone in the area of the substitution; 2) the charge or hydrophobicity of the molecule at the target site; or, 3) the bulk of the side chain. Additionally, in many cases, alterations of the N-terminal and C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein.

[0080] The term "non-conservative amino acid substitution" refers to an amino acid substitution that is used to produce the greatest change in protein properties. Thus, for example, a non-conservative amino acid substitution would be one whereby: 1) a hydrophilic residue is substituted for/by a hydrophobic residue (e.g., Ser or Thr for/by Leu, Ile, Val); 2) a Cys or Pro is substituted for/by any other residue; 3) a residue having an electropositive side chain is substituted for/by an electronegative residue (e.g., Lys, Arg or His for/by Asp or Glu); or 4) a residue having a bulky side chain is substituted for/by one not having a side chain (e.g., Phe for/by Gly). Sometimes, non-conservative amino acid substitutions between two of the five groups will not affect the activity of the encoded protein.

[0081] The term "silent mutation" refers to a mutation in a DNA sequence that does not result in an amino acid change in the encoded polypeptide. These mutations often occur as a result of the degeneracy of the genetic code, wherein more than one codon may specify an amino acid. For example, TCT, TCA, TCG and TCC all encode the amino acid Ser; thus, a TCT to TCA mutation in the DNA sequence will only be detected by sequencing the gene (or its mRNA), since there is no alteration in the amino acid in the synthesized protein.

[0082] The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", "nucleic acid molecule", "nucleic acid fragment" and "isolated nucleic acid fragment" are used interchangeably herein. As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0083] As used herein, a nucleic acid fragment is "hybridizable" to another nucleic acid fragment, such as a cDNA, genomic DNA, or RNA molecule, when a single-stranded form of the nucleic acid fragment can anneal to the other nucleic acid fragment under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989), particularly Chapter 11 and Table 11.1.

[0084] A "substantial portion" of an amino acid or nucleotide sequence is that portion comprising enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using

algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., J. Mol. Biol., 215:403-410 (1993)). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to identify putatively a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation, such as *in situ* hybridization of bacterial colonies or bacteriophage plaques. In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence. The disclosure herein teaches the complete amino acid and nucleotide sequences encoding particular LPCATs and PDATs. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art.

[0085] The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine.

[0086] The terms "homology", "homologous", "substantially similar" and "corresponding substantially" are used interchangeably herein. They refer to nucleic acid fragments or polypeptides that have similar, but not identical sequences These terms sometimes also refer to modifications of the nucleic acid fragments (e.g., via deletion or insertion of one or more nucleotides) that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences.

[0087] "Sequence identity" or "identity" in the context of nucleic acid or polypeptide sequences refers to the nucleic acid bases or amino acid residues in two sequences that are the same when aligned for maximum correspondence over a specified comparison window

[0088] Thus, "percentage of sequence identity" or "percent identity" refers to the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the results by 100 to yield the percentage of sequence identity.

[0089] Methods to determine "percent identity" and "percent similarity" are codified in publicly available computer programs. Percent identity and percent similarity can be readily calculated by known methods, including but not limited to those described in: 1) Computational Molecular Biology (Lesk, A. M., Ed.) Oxford University: NY (1988); 2) Biocomputing: Informatics and Genome Projects (Smith, D. W., Ed.) Academic: NY (1993); 3) Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., Eds.) Humana: NJ (1994); 4) Sequence Analysis in Molecular Biology (von Heinje, G., Ed.) Academic (1987); and, 5) Sequence Analysis Primer (Gribskov, M. and Devereux, J., Eds.) Stockton: NY (1991).

[0090] Sequence alignments and percent identity or similarity calculations may be determined using a variety of comparison methods designed to detect homologous sequences including, but not limited to, the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences is performed using the "Clustal method of alignment" which encompasses several varieties of the algorithm including the "Clustal V method of alignment" and the "Clustal W method of alignment" (described by Higgins and Sharp, CABIOS, 5:151-153 (1989); Higgins, D.G. et al., Comput. Appl. Biosci., 8:189-191 (1992)) and found in the MegAlign™ (version 8.0.2) program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.). Default parameters for multiple protein alignment using the Clustal W method of alignment correspond to GAP PENALTY=10, GAP LENGTH PENALTY=0.2, Delay Divergent Seqs(%)=30, DNA Transition Weight=0.5, Protein Weight Matrix=Gonnet Series, DNA Weight Matrix=IUB with the 'slow-accurate' option. After alignment of the sequences using either Clustal program, it is possible to obtain a "percent identity" by viewing the "sequence distances" table in the program.

[0091] It is well understood by one skilled in the art that many levels of sequence identity are useful in identifying polypeptides, from other species, wherein such polypeptides have the same or similar function or activity. Suitable nucleic acid fragments, i.e., isolated polynucleotides according to the disclosure herein, encode polypeptides that are at least about 70-85% identical, while more preferred nucleic acid fragments encode amino acid sequences that are at least about 85-95% identical to the amino acid sequences reported herein. Although preferred ranges are described above, useful examples of amino acid sequence percent identities include any integer percentage from 45% to 100%, such as 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. Also, of interest is any full-length or partial complement of this isolated nucleotide fragment.

[0092] Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids.

[0093] "Codon degeneracy" refers to the nature in the genetic code permitting variation of the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

[0094] "Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These oligonucleotide building blocks are annealed and then ligated to form gene segments that are then enzymatically assembled to construct the entire gene. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell, where sequence information is available. For example, the codon usage profile for *Yarrowia lipolytica* is provided in U.S. Patent 7,125,672.

[0095] "Gene" refers to a nucleic acid sequence that expresses a specific protein, and that may refer to the coding region alone or may include regulatory sequences upstream and/or downstream to the coding region (e.g., 5'-untranslated regions upstream of the transcription start site of the coding region, 3' non-coding regions). "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature (i.e., heterologous with respect to each other). Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, native genes introduced into a new location within the native host, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. A "codon-optimized gene" is a gene having its frequency of codon usage designed to mimic the frequency of preferred codon usage of the host cell.

[0096] "Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream of the coding sequence's transcription start site, 5'-untranslated regions and 3' non-coding regions, and which may influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include, but are not limited to: promoters, enhancers, silencers, 5'-untranslated leader sequence, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, stem-loop structures and terminators.

[0097] "Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". It is further recognized that since in most cases the exact boundaries of regulatory sequences (especially at their 5' end) have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

[0098] The terms "3' non-coding sequences", "transcription terminator", "terminator" and "termination sequences" refer to DNA sequences located 3' downstream of a coding sequence. This includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3'-end of the mRNA precursor. The 3' region can influence the transcription, RNA processing or stability, or translation of the associated coding sequence.

[0099] "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA" or "mRNA" refers to the RNA that is without introns and which can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to, and derived from, mRNA.

[0100] The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of

affecting the expression of that coding sequence. That is, the coding sequence is under the transcriptional control of the promoter. Regulatory sequences can be operably linked to coding sequences in sense or antisense orientation.

[0101] The term "recombinant" or "heterologous" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

[0102] The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA. Expression may also refer to translation of mRNA into a protein (either precursor or mature).

[0103] "Transformation" refers to the transfer of a nucleic acid molecule into a host organism, resulting in genetically stable inheritance. The nucleic acid molecule may be a plasmid that replicates autonomously, for example, or it may integrate into the genome of the host organism. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" or "transformant" organisms.

[0104] The terms "plasmid" and "vector" refer to an extrachromosomal element often carrying genes that are not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA fragments. Such elements may have autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, and may be linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction that is capable of introducing an expression cassette(s) into a cell.

[0105] The term "expression cassette" refers to a fragment of DNA containing a foreign gene and having elements in addition to the foreign gene that allow for expression of that gene in a foreign host. Generally, an expression cassette will comprise the coding sequence of a selected gene and regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence that are required for expression of the selected gene product. Thus, an expression cassette is typically composed of: 1) a promoter sequence; 2) a coding sequence (i.e., ORF); and 3) a terminator that usually contains a polyadenylation site in eukaryotes. The expression cassette(s) is usually included within a vector to facilitate cloning and transformation. Different expression cassettes can be transformed into different organisms including bacteria, yeast, plants and mammalian cells, as long as the correct regulatory sequences are used for each host.

[0106] The terms "recombinant construct", "expression construct", "chimeric construct", "construct", and "recombinant DNA construct" are used interchangeably herein. A recombinant construct comprises an artificial combination of nucleic acid fragments, e.g., regulatory and coding sequences that are not found together in nature. For example, a recombinant DNA construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. Such a construct may be used by itself or may be used in conjunction with a vector. If a vector is used, then the choice of vector is dependent upon the method that will be used to transform host cells as is well known to those skilled in the art. For example, a plasmid vector can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells comprising any of the isolated nucleic acid fragments described herein. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., EMBO J., 4:2411-2418 (1985); De Almeida et al., Mol. Gen. Genetics, 218:78-86 (1989)), and thus that multiple events must be screened in order to obtain strains or lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, northern analysis of mRNA expression, western and/or ELISA analyses of protein expression, formation of a specific product, phenotypic analysis or GC analysis of the PUFA products, among others.

[0107] The terms "host cell" and "host organism" are used interchangeably herein and refer to any organism such as a microorganism or a plant (e.g., an oilseed plant) that is capable of receiving foreign or heterologous genes and capable of expressing those genes. A "recombinant host cell" refers to a host cell that has been recombinantly engineered.

[0108] Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989); by Silhavy, T. J., Bennan, M. L. and Enquist, L. W., Experiments with Gene Fusions, Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1984); and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience, Hoboken, NJ (1987).

[0109] In a first embodiment, described herein is a recombinant *Yarrowia* cell for the production of at least one long-chain (LC) polyunsaturated fatty acid (PUFA) having a chain length of C20 or greater, wherein said recombinant cell has been genetically engineered to comprise a polyunsaturated fatty acid biosynthetic pathway capable of producing at least one long-chain polyunsaturated fatty acid, and has been further modified to introduce:

1. (a) a chimeric gene encoding at least one polypeptide having LPCAT activity wherein said polypeptide has at least 90% amino acid identity, based on the Clustal W method of alignment, when compared to the amino acid sequence set forth in SEQ ID

NO: 4 (YILPCAT); and

2. (b) a chimeric gene encoding at least one polypeptide having PDAT activity wherein said polypeptide has at least 90% amino acid identity, based on the Clustal W method of alignment, when compared to the amino acid sequence of SEQ ID NO: 32 (YIPDAT); and

wherein said chimeric genes each comprise a promoter which is heterologous to the coding sequence encoding the polypeptides of (a) and (b), and wherein the recombinant cell comprises an increased amount of a said polyunsaturated fatty acid measured as a weight percent of total fatty acids ["wt. % TFAs"], when compared to a control cell which corresponds to the recombinant *Yarrowia* cell for production but which has not been modified to introduce the chimeric genes encoding the LPCAT or PDAT polypeptides of (a) and (b).

[0110] Over-expression of PDAT and LPCAT can be achieved, for example, by introducing polynucleotides encoding these enzymes (i.e., transgenes) to cells. Preferably, such polynucleotides are operably linked to a regulatory sequence such as a promoter that allows gene expression in the cells modified to contain the polynucleotides. Over-expression of PDAT and LPCAT is with respect to the expression of PDAT and LPCAT in a control cell.

[0111] An increase in the amount of the at least one long-chain PUFA (e.g., EPA) measured as a weight percent of total fatty acids ["wt. % TFAs"] of the recombinant microbial cell over-expressing PDAT and LPCAT may be at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11 %, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% over the amount of the at least one long-chain PUFA measured as a weight percent of total fatty acids of a control cell.

[0112] With respect to over-expressing PDAT and LPCAT, a control cell, corresponding control cell, or suitable control cell may be a wild type or recombinant cell that corresponds to the recombinant microbial cell, but does not comprise the over-expressed PDAT and LPCAT polypeptides. For example, the control cell does not over-express the PDAT and LPCAT polypeptides by virtue of not comprising recombinant polynucleotide sequences encoding the PDAT and LPCAT polypeptides. Also for example, the control cell does not over-express the PDAT and LPCAT polypeptides by virtue of comprising, but not expressing, recombinant polynucleotide sequences encoding the PDAT and LPCAT polypeptides. The control cell may be the recombinant microbial cell as it existed before it was modified to over-express the PDAT and LPCAT polypeptides (i.e., a parent cell), or may be a recombinant microbial cell that has been modified to contain the recombinant polynucleotides encoding PDAT and LPCAT, but does not over-express the recombinant PDAT and LPCAT polypeptides (e.g., a cell prepared in parallel with the recombinant microbial cell that over-expresses the PDAT and LPCAT polypeptides).

[0113] PDAT catalyzes TAG biosynthesis by transferring an acyl group from the *sn*-2 position of phospholipids such as phosphatidylcholine ["PC"], phosphatidylethanolamine ["PE"], and phosphatidic acid ["PA"] to the *sn-3* position of 1,2-diacylglycerol ["DAG"]. This reaction results in lysophospholipids such as lysophosphatidylcholine ["LPC"], lysophosphatidylethanolamine ["LPE"], lysophosphatidic acid ["LPA"] and lysophosphatidylglycerol ["LPG"]. LPCAT can regenerate PC by transferring an acyl group from acyl-CoA to the *sn*-2 position of its substrate LPC. Fatty acid remodeling may occur in this manner, since PC_1 (FIG. 1) may not be equivalent to PC_2, depending on which fatty acid from the acyl-CoA pool is used to replace the fatty acid that was removed by PDAT. This cycle of PC substrate use (PC_1) by PDAT and regeneration (PC_2) by LPCAT is diagrammed in FIG. 1.

[0114] While the recombinant microbial cell over-expressing LPCAT and PDAT produces an increased amount of long-chain polyunsaturated fatty acid measured as a wt. % TFAs when compared to a control cell, the recombinant microbial cell may also have: (i) an increased C_{18} to C_{20} elongation conversion efficiency; and/or (ii) an increased total lipid content (i.e., the amount of total fatty acids, measured as a weight percent of the dry cell weight ["TFAs % DCW"]), compared to a control cell.

[0115] The increased C_{18} to C_{20} elongation conversion efficiency may be either the effect of increased delta-9 elongase conversion efficiency, i.e., when the recombinant microbial cell's PUFA biosynthetic pathway comprises a delta-9 elongase, and/or the effect of increased delta-6 elongase conversion efficiency, i.e., when the recombinant microbial cell's PUFA biosynthetic pathway comprises a delta-6 elongase. The increase in the C_{18} to C_{20} elongation conversion efficiency, delta-9 elongase conversion efficiency, and/or delta-6 elongase conversion efficiency of the recombinant microbial cell over-expressing PDAT and LPCAT may be at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% over the C_{18} to C_{20} elongation conversion efficiency, delta-9 elongase conversion efficiency, and/or delta-6 elongase conversion efficiency, respectively, of a control cell.

[0116] Total lipid content ["TFAs % DCW"] may be increased in the recombinant microbial cell over-expressing LPCAT and PDAT. As is well known to one of skill in the art, economical commercial production of a LC polyunsaturated fatty acid in a recombinant microbial host cell requires consideration of a variety of variables, including the LC polyunsaturated fatty acid concentration ["LC polyunsaturated fatty acid % TFAs"], total lipid content ["TFAs % DCW"] and LC polyunsaturated fatty acid productivity ["LC polyunsaturated fatty acid % DCW"]. Selection of a preferred strain for commercial purposes will consider both the LC polyunsaturated fatty acid % TFAs) and TFAs % DCW, as both factors affect the cellular content of the LC polyunsaturated fatty acid as a percent of the dry cell weight.

[0117] The increase in the total lipid content (TFAs % DCW) of the recombinant microbial cell over-expressing PDAT and LPCAT may be at least about 1%, 2%, 3%, 4%, or 5% over the total lipid content of a control cell. The increase in total lipid content can coincide with an increase in EPA % TFAs.

[0118] The recombinant microbial cells of the present invention over-express at least one polypeptide having PDAT activity. Dahlqvist et al. (Proc. Natl. Acad. Sci. U.S.A., 97:6487-6492 (2000)) and Oelkers et al. (J. Biol. Chem., 275:15609-15612 (2000)) were the first to appreciate that TAG synthesis can occur in the absence of acyl-CoA, via the acyl-CoA-independent PDAT enzyme (structurally related to the lecithin:cholesterol acyltransferase family of proteins). More specifically, Dahlqvist et al. and Oelkers et al. demonstrated that overexpression of the *Saccharomyces cerevisiae* LRO1 gene encoding PDAT (SEQ ID NO:30; "ScPDAT") resulted in an increased TAG content, while deletion of ScPDAT caused significant reduction of TAG synthesis. Following this work, U.S. Pat. No. 7,267,976 described the cloning, overexpression and knockout of the *Yarrowia lipolytica* ATCC #90812 gene encoding PDAT (SEQ ID NOs:31 and 32 herein), which was determined to share 47.1% amino acid sequence identity with ScPDAT. Y. *lipolytica* strains having disrupted PDAT were found to have lower oil content ["TFAs % DCW"] as compared to the wild type strain (ca. 29-38%), while strains having a disruption in both PDAT2 and DGAT2 were determined to have only 17-27% oil content when compared to the control. The *Y. lipolytica* PDAT was then expressed in an *S. cerevisiae* strain having a disruption in its native PDAT and DGAT2 genes; TFAs % DCW was doubled in the transformant strains as compared to the control.

[0119] For purposes herein, a polypeptide having PDAT activity may be selected from the group consisting of: (a) a sequence consisting essentially of a sequence selected from the group consisting of SEQ ID NO:32; and (b) a polypeptide having at least 90% or 95% amino acid identity, based on the Clustal W method of alignment, when compared to an amino acid sequence of SEQ ID NO:32. In this sense, the polypeptide having PDAT activity may be derived from a yeast for example; preferably the yeast PDAT polypeptide is derived from *Yarrowia lipolytica*.

[0120] One of skill in the art will appreciate that either of the sequences set forth as SEQ ID NOs:30 and 32, or portions thereof, may be used to search for PDAT homologs in the same or other algal, fungal, oomycete, euglenoid, stramenopiles, yeast or plant species using sequence analysis software. In general, such computer software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Use of software algorithms, such as the BLASTP method of alignment with a low complexity filter and the following parameters: Expect value = 10, matrix = Blosum 62 (Altschul, et al., Nucleic Acids Res., 25:3389-3402 (1997)), is well-known for comparing any PDAT protein against a database of nucleic or protein sequences and thereby identifying similar known sequences within a preferred host organism.

[0121] Alternatively, publicly available PDAT sequences or their motifs may be hybridization reagents for the identification of homologs. Hybridization methods are well known to those of ordinary skill in the art as noted above.

[0122] Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to: 1) methods of nucleic acid hybridization; 2) methods of DNA and RNA amplification, as exemplified by various uses of nucleic acid amplification technologies, such as polymerase chain reaction ["PCR"] (U.S. Pat. No. 4,683,202); ligase chain reaction ["LCR"] (Tabor et al., Proc. Natl. Acad. Sci. U.S.A., 82:1074 (1985)); or strand displacement amplification ["SDA"] (Walker et al., Proc. Natl. Acad. Sci. U.S.A., 89:392 (1992)); and 3) methods of library construction and screening by complementation.

[0123] Based on well-known methods available to one of skill in the art, it would be possible to identify and/or isolate PDAT gene homologs in any preferred eukaryotic organism of choice. The activity of any putative PDAT gene can readily be confirmed by expression of the gene within a LC-PUFA-producing host organism, since the LC-polyunsaturated fatty acids measured as a wt. % of TFAs are increased (when co-expressed with a suitable PDAT) relative to those within a control not over-expressing the LPCAT and PDAT transgenes.

[0124] The recombinant microbial cells of the present invention over-express at least one polypeptide having LPCAT activity, wherein the polypeptide can be a wild type protein or a mutant protein that is synthetically created (i.e., not naturally occurring). This polypeptide has at least 90% amino acid identity, based on the Clustal W method of alignment, when compared to the amino acid sequence SEQ ID NO:4 (YlLPCAT). The polypeptide may further be selected from the group consisting of:

1. (a) a polypeptide comprising at least one membrane bound O-acyltransferase protein family motif selected from the group consisting of: SEQ ID NO:5 (WHG-X_3-GY-X_3-F), SEQ ID NO:6 (Y-X_4-F), SEQ ID NO:7 (Y-X_3-YF-X_2-H), SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG), SEQ ID NO:9 (RxKYY-X_2-W-X_3-[E/D]-[A/G]-X_5-GxG-[F/Y]-xG), SEQ ID NO:10 (EX_{11}WN-X_2-[T/V]-X_2-W), SEQ ID NO:11 (SAxWHG-X2-PGY-X2-[T/F]-F), SEQ ID NO:12 (M-[V/I]-[L/I/V]-[V/C/A/T]-[M/L/Q]-K-[L/V/I/M]-[S/T/Y/I]-[S/T/A/M/G]-[F/L/C/Y]-[C/A/G/S]-[W/Y/M/I/F/C]-[N/S/E/Q/D]-[V/Y/L/I]-[H/Y/A/N/S/T]-DG), SEQ ID NO:13 (R-[L/M/F/W/P/Y]-KYY-[G/A/F/H/S]-[V/A/I/C]-W-[Y/E/T/M/S/L]-[L/I/N]-[T/S/A]-[E/D]-[G/A]-[A/S/I/V]-[C/S/I/N/H/L]-[V/I/N]-[L/I/N/A/C]-[S/C/W/A/I]-G-[M/I/L/A/F]-G-[Y/F]-[N/E/S/T/R/K]-G), SEQ ID NO:14 (E-[T/F/L/M]-[A/S]-[Q/D/P/K/T]-[N/S]-[S/I/T/L/A/M/F]-[H/K/R/V]-[G/C/E/T/Q/D/M]-[Y/A/M/L/I/F]-[L/S/P/I]-[G/E/A/L/N/D]-[S/A/V/F/M/N]-WN-[K/M/I/C]-[N/K/Q/G]-[T/V]-[N/A/S]-[H/K/N/T/R/L]-W), SEQ ID NO:15 (SA-[F/M/V/I]-WHG-[F/V/T/L]-[Y/S/R]-PGY-[Y/M/I]-[L/M/I/F]-[T/F]-F), SEQ ID NO:16 (M-[V/I]-L-X_2-KL), SEQ ID

NO:17 (RxKYY-X_2-W), and SEQ ID NO:18 (SAxWHG);

2. (b) a polypeptide comprising at least one mutant membrane bound O-acyltransferase protein family motif.

[0125] The polypeptide having LPCAT activity may be derived from a yeast for example; preferably the yeast LPCAT polypeptide is derived from *Saccharomyces cerevisiae* or *Yarrowia lipolytica.*

[0126] Either the LPCAT sequences set forth herein as SEQ ID NO:2 [ScLPCAT] and SEQ ID NO:4 [YlLPCAT], or portions thereof, or the LPAATs set forth herein as SEQ ID NO:24 [ScLPAAT], SEQ ID NO:21 [MaLPAAT1] and SEQ ID NO:23 [YlLPAAT1], or portions of them, may be used to search for LPCAT homologs in the same or other species using sequence analysis software, as described above with respect to PDATs.

[0127] Use of a software algorithm to comb through databases of known sequences is particularly suitable for the isolation of homologs having a relatively low percent identity to publicly available LPCAT sequences, such as those described in SEQ ID NOs:2 and 4. It is predictable that isolation would be relatively easier for LPCAT homologs of at least about 70%-85% identity to publicly available LPCAT sequences. Further, those sequences that are at least about 85%-90% identical would be particularly suitable for isolation and those sequences that are at least about 90%-95% identical would be the most facilely isolated.

[0128] LPCAT homologs can also be identified by the use of motifs unique to the LPCAT enzymes, e.g., membrane bound O-acyltransferase ["MBOAT"] family motifs such as described in Table 2. LPCATs that have both LPCAT and LPAAT activity may also be identified by the use of motifs unique to the LPAAT enzymes, e.g., 1-acyl-*sn*-glycerol-3-phosphate acyltransferase family motifs selected from the group consisting of: NHxxxxD (SEQ ID NO:25) and EGTR (SEQ ID NO:26).

[0129] Based on well-known methods available to one of skill in the art, it would be possible to identify and/or isolate LPCAT gene homologs in any preferred eukaryotic organism of choice. The activity of any putative LPCAT gene can readily be confirmed by expression of the gene within a LC-PUFA-producing host organism, since the LC-PUFAs, measured as a wt. % of TFAs, are increased (when co-expressed with a suitable PDAT) relative to those within an organism not over-expressing both the LPCAT and PDAT transgenes (above).

[0130] Considerable effort was invested toward the identification of an isolated polynucleotide encoding a non-naturally occurring mutant polypeptide having LPCAT activity, wherein said mutant polypeptide comprises at least one mutant membrane-bound O-acyltransferase protein motif, said mutant motif selected from the group consisting of:

1. (a) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:33, wherein SEQ ID NO:33 differs from SEQ ID NO:16 (M-[V/I]-L-X_2-KL) by at least one amino acid mutation, said mutation selected from the group consisting of: M1A, M1N, M1C, M1G, M1Q, M1H, M1I, M1L, M1F, M1P, M1S, M1T, M1W, M1Y, M1V, V2A, V2N, V2C, V2G, V2Q, V2H, V2L, V2M, V2F, V2P, V2S, V2T, V2W, V2Y, I2A, I2N, I2C, I2G, I2Q, I2H, I2L, I2M, I2F, I2P, I2S, I2T, I2W, I2Y, L3A, L3N, L3C, L3G, L3Q, L3H, L3M, L3F, L3P, L3S, L3T, L3W, L3Y, L3V, K6A, K6R, K6N, K6G, K6H, K6P, K6S, K6T, K6Y, L7A, L7N, L7C, L7G, L7Q, L7H, L7I, L7M, L7F, L7P, L7S, L7T, L7W and L7Y;
2. (b) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:34, wherein SEQ ID NO:34 differs from SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG) by at least amino acid mutation, said mutation selected from the group consisting of: M1A, M1 N, M1C, M1G, M1Q, M1H, M1I, M1L, M1F, M1P, M1S, M1T, M1W, M1Y, M1V, V2A, V2N, V2C, V2G, V2Q, V2H, V2L, V2M, V2F, V2P, V2S, V2T, V2W, V2Y, I2A, I2N, I2C, I2G, I2Q, I2H, I2L, I2M, I2F, I2P, I2S, I2T, I2W, I2Y, L3A, L3N, L3C, L3G, L3Q, L3H, L3M, L3F, L3P, L3S, L3T, L3W, L3Y, L3V, I3A, I3N, I3C, I3G, I3Q, I3H, I3M, I3F, I3P, I3S, I3T, I3W, I3Y, I3V, K6A, K6R, K6N, K6G, K6H, K6P, K6S, K6T, K6Y, L7A, L7N, L7C, L7G, L7Q, L7H, L7I, L7M, L7F, L7P, L7S, L7T, L7W, L7Y, V7A, V7N, V7C, V7G, V7Q, V7H, V7I, V7M, V7F, V7P, V7S, V7T, V7W, V7Y, I7A, I7N, I7C, I7G, I7Q, I7H, I7M, I7F, I7P, I7S, I7T, I7W, I7Y, D16A, D16N, D16G, D16E, D16Q, D16H, D16F, D16S, D16T, G17A, G17N, G17H, G17L, G17M, G17F, G17S, G17T and G17V;
3. (c) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:35, wherein SEQ ID NO:35 differs from SEQ ID NO:5 (WHG-X_3-GY-X_3-F) by at least one amino acid mutation, said mutation selected from the group consisting of: G7A, G7N, G7C, G7H, G7I, G7L, G7K, G7M, G7F, G7S, G7T, G7W, G7Y, G7V, Y8A, Y8G, Y8H, Y8L, Y8F, Y8P, Y8S, Y8T, Y8V, F12A, F12N, F12C, F12G, F12H, F12L, F12M, F12P, F12S, F12T and F12V;
4. (d) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:36, wherein SEQ ID NO:36 differs from SEQ ID NO:11 (SAxWHG-X_2-PGY-X_2-[T/F]-F) by at least one amino acid mutation, said mutation selected from the group consisting of: S1A, S1G, S1H, S1L, S1F, S1P, S1T, S1V, A2N, A2G, A2H, A2L, A2F, A2P, A2S, A2T, A2V, P9A, P9R, P9G, P9H, P9I, P9L, P9K, P9M, P9F, P9S, P9T, P9W, P9Y, P9V, G10A, G10N, G10C, G10H, G10I, G10L, G10K, G10M, G10F, G10S, G10T, G10W, G10Y, G10V, Y11A, Y11G, Y11H, Y11L, Y11F, Y11P, Y11S, Y11T, Y11V, T14A, T14C, T14G, T14H, T14I, T14L, T14M, T14F, T14P, T14S, T14W, T14Y, T14V, F14A, F14C, F14G, F14H, F14I, F14L, F14M, F14P, F14S, F14W, F14Y, F14V, F15A, F15N, F15C, F15G, F15H, F15L, F15M, F15P, F15S, F15T and F15V; and
5. (e) a complement of the nucleotide sequence of part (a), (b), (c) or (d), wherein the complement and the nucleotide sequence

consist of the same number of nucleotides and are 100% complementary.

[0131] Therefore, disclosed herein is an isolated polynucleotide encoding a mutant polypeptide having acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) activity, wherein the mutant polypeptide comprises at least one mutant membrane-bound *O*-acyltransferase protein motif, and the polynucleotide is operably linked to at least one regulatory sequence.

[0132] For example, the polynucleotide may encode a mutant yeast (e.g., *Yarrowia*) LPCAT polypeptide having a mutation in Motif I and/or Motif II. Alternatively, the polynucleotide may encode an amino acid sequence that has LPCAT activity and that is at least 90%, or 95%, identical to SEQ ID NO:4 (wild type YlLPCAT) based on the Clustal W method of alignment, and that has one or more mutations (e.g., amino acid substitution, deletion, and/or insertion) in Motif I (SEQ ID NO:4 residues 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148) and/or Motif II (SEQ ID NO:4 residues 376, 377, 378, 382, 383, 384, 385, 386, 387, 389, 390). Substitution mutations may be any of those described herein, for example. Preferably, the activity of a mutant LPCAT polypeptide encoded by a polynucleotide is equal to or greater than the activity of wild type YlLPCAT (e.g., SEQ ID NO:4). Such activity can be determined by comparing the EPA % TFAs and/or d9e CE(%) in recombinant cells (e.g., microbial cells) over-expressing a mutant LPCAT with the EPA % TFAs and/or d9e CE(%) in a control cell.

[0133] As another example, the polynucleotide may encode a polypeptide that has LPCAT activity and that is at least 90% or 95% identical to: SEQ ID NO:79, where the polypeptide has a serine at position 136 and an alanine at position 389; SEQ ID NO:81, where the polypeptide has a serine at position 136 and a cysteine at position 389; SEQ ID NO:83, where the polypeptide has a serine at position 136 and a serine at position 389; SEQ ID NO:85, where the polypeptide has a valine at position 136 and a cysteine at position 389; SEQ ID NO:87, where the polypeptide has an alanine at position 144 and a serine at position 390; SEQ ID NO:89, where the polypeptide has an alanine at position 148 and a serine at position 390; SEQ ID NO:91, where the polypeptide has an asparagine at position 148 and an isoleucine at position 382; or SEQ ID NO:93, where the polypeptide has an asparagine at position 148 and a serine at position 390.

[0134] Methods for synthesizing sequences and bringing sequences together are well established in the literature. Many techniques are commonly employed to obtain mutations of naturally occurring genes (wherein such mutations may include deletions, insertions and point mutations, or combinations thereof). The present work was conducted with the goal of identifying suitable mutation(s) within an LPCAT (e.g., YlLPCAT [e.g., SEQ ID NO:4]) that would be tolerated within the enzyme when it was expressed in a microbial cell engineered to produce at least one LC-polyunsaturated fatty acid. More preferably, identification of mutations that increased the amount of LC-polyunsaturated fatty acid, measured as a wt. % of TFAs, and/or the C_{18} to C_{20} elongation conversion efficiency was especially desirable as a means to increase the overall rate and quantity of PUFA biosynthesis.

[0135] A variety of LPCAT mutations are described herein within two specific conserved motifs within the *Yarrowia lipolytica* LPCAT polypeptide. Specifically, a suite of site-saturation libraries were created within the 17 amino acid residues within Motif I, corresponding to SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V//I]-X_8-DG) and within 12 of the 15 amino acid residues of Motif II, corresponding to SEQ ID NO:11 (SAxWHG-X2-PGY-X2-[T/F]-F), using YlLPCAT (SEQ ID NO:4) as a template, wherein YlLPCAT was contained within a plasmid construct comprising a chimeric YAT1::YlLPCAT::Lip1 gene. The site-saturation libraries, each comprising a single amino acid change with respect to the YlLPCAT polypeptide, were then transformed into *Yarrowia lipolytica*, and screened for improved delta-9 elongase conversion efficiency ["% Conv."] (i.e., based on conversion of C18 PUFAs to C20 PUFAs) and/or improved production of EPA as a weight percent of TFAs ["EPA % TFAs"] based on GC analyses. These indirect means were utilized to analyze LPCAT activity, as opposed to a direct method.

[0136] More specifically, amino acid residues 132 to 148 (Motif I) and amino acid residues 376 to 378 and 382 to 390 (Motif II) within YlLPCAT were individually mutated. All 329 of the mutants performed such that the EPA % TFAs was at least 75% of that of the control YlLPCAT polypeptide; and all of the mutants performed with a % Conv. that was at least 87.6% of that of the control YlLPCAT polypeptide. Fifty-six (56) YlLPCAT mutants were found to exhibit equivalent or improved EPA % TFAs and equivalent or improved % Conv. An additional 14 YlLPCAT* mutants were determined to have an equivalent or improved EPA % TFAs when compared to the control (but did not have an equivalent or improved % Conv.); an additional 12 YlLPCAT mutants were determined to have an equivalent or improved % Conv., when compared to the control (but did not have an equivalent or improved EPA % TFAs). Thus, this work demonstrated that the LPCAT activity of YlLPCAT could indeed be modified without negative implications and even improved by protein engineering.

[0137] Mutants resulting in equivalent or improved LPCAT activity were generated at amino acid residues 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147 and 148 within Motif I, thereby demonstrating that only the methionine [M] residue of SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG) appears unable to tolerate variation. Similarly, mutants resulting in equivalent or improved LPCAT activity were generated at amino acid residues 378, 382, 383, 385, 388, 389 and 390 within Motif II, thereby demonstrating that the serine [S], alanine [A], proline [P] and tyrosine [Y] of SEQ ID NO:11 (SAxWHG-X2-PGY-X2-[T/F]-F) appear unable to tolerate variation. The amino acids at residues 379-381, (i.e., WHG) were not subjected to mutation, since the histidine of

other LPCATs corresponding to H380 of YILPCAT has been reported to be a likely active site residue (Lee et al., 2008, Mol. Biol. Cell 19:1174-1184).

[0138] Thus, disclosed herein is an isolated polynucleotide encoding a non-naturally occurring mutant polypeptide having lysophosphatidylcholine acyltransferase ["LPCAT"] activity comprising at least one mutant membrane bound *O*-acyltransferase protein motif, wherein:

1. (a) the mutant polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:19, wherein SEQ ID NO:19 differs from SEQ ID NO:4 (YILPCAT) by at least one amino acid mutation, wherein:
    1. (i) the amino acid mutation is an amino acid substitution at a residue selected from the group consisting of: residue 133, residue 134, residue 135, residue 136, residue 137, residue 138, residue 139, residue 140, residue 141, residue 142, residue 143, residue 144, residue 145, residue 146, residue 147 and residue 148;
    2. (ii) the amino acid mutation is in an amino acid substitution at a residue selected from the group consisting of: residue 378, residue 382, residue 383, residue 385, residue 388, residue 389 and residue 390; or
    3. (iii) there are at least two amino acid mutations, wherein:
        1. (1) a first amino acid mutation is an amino acid substitution selected from the group set forth in part (i), and
        2. (2) the second amino acid mutation is an amino acid substitution selected from the group set forth in part (ii);
2. (b) overexpression of the mutant polypeptide in a recombinant *Yarrowia* cell comprising a polyunsaturated fatty acid biosynthetic pathway that is capable of producing at least one long-chain polyunsaturated fatty acid produces a result selected from the group consisting of:
    1. (i) an amount of at least one long-chain polyunsaturated fatty acid, measured as a weight percent of total fatty acids that is at least the same as or greater than the amount produced by a control *Yarrowia* cell; and
    2. (ii) a C_{18} to C_{20} elongation conversion efficiency that is at least the same as or greater than the conversion efficiency of a control *Yarrowia* cell.

[0139] Mutant polypeptides having LPCAT activity encoded by the isolated polynucleotide described above are also disclosed herein.

[0140] The amino acid sequence of a mutant YILPCAT polypeptide may comprise an amino acid sequence as set forth in SEQ ID NO:37, wherein SEQ ID NO:37 differs from SEQ ID NO:4 (YILPCAT) and wherein said difference is an amino acid mutation selected from the group consisting of: L134A, L134C, L134G, C135D, C135I, M136G, M136P, M136S, M136V, K137N, K137G, K137H, K137Y, L138A, L138H, L138M, S139L, S139W, S140N, S140H, S140P, S140W, F141A, F141M, F141W, G142H, W143L, N144A, N144K, N144F, N144T, N144V, V145A, V145G, V145E, V145M, V145F, V145W, Y146G, Y146L, Y146M, D147N, D147Q, D147H, G148A, G148N, T382I, T382P, R383M, L388G, L388Y, T389A, T389C, T389S, F390C, V133C, M136N, L138G, L138I, L138N, S139G, S139N, W143H, G148V, L388H, L388T, F390G, F390N, F390T, C135F, M136T, S140Y, S140I, F141V, G142I, G142V, D147E, F378Y, T382Y, R383A and F390S.

[0141] More specifically, and of applicability for use in any recombinant microbial cell (e.g., wherein said LC-polyunsaturated product fatty acid-producing cell is over-expressing both a PDAT and LPCAT), also described herein is a polypeptide having LPCAT activity comprising at least one mutant membrane bound *O*-acyltransferase protein motif, wherein the mutant motif is selected from the group consisting of:

1. (a) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:38, wherein SEQ ID NO:38 differs from SEQ ID NO:16 (M-[V/I]-L-X_2-KL) by at least one amino acid mutationselected from the group consisting of: V2C, 12C, L3A, L3C, L3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7I and L7M;
2. (b) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:39, wherein SEQ ID NO:39 differs from SEQ ID NO:8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG) by at least one amino acid mutation selected from the group consisting of: V2C, I2C, L3A, L3C, L3G, I3A, I3C, I3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7I, L7M, V7A, V7N, V7G, V7H, V7M, I7A, I7N, I7G, I7H, I7M, D16Q, D16N, D16H, G17A, G17V and G17N;
3. (c) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:40 wherein SEQ ID NO:40 differs from SEQ ID NO:5 (WHG-X_3-GY-X_3-F) by at least one amino acid mutation selected from the group consisting of: F12N, F12C, F12G, and F12T; and
4. (d) a mutant motif comprising an amino acid sequence as set forth in SEQ ID NO:41, wherein SEQ ID NO:41 differs from SEQ ID NO:11 (SAxWHG-X_2-PGY-X_2-[T/F]-F) by at least one amino acid mutation selected from the group consisting of: T14A, T14C, T14S, F15N, F15C, F15G and F15T.

[0142] The specific mutations set forth above correspond to mutations identified within YILPCAT according to the methodologies described above, and that were demonstrated to result in mutants having equivalent or improved EPA % TFAs and/or equivalent or

improved % Conv.

[0143] Following the work set forth above, wherein single amino acid mutations were created within either Motif I or Motif II of YILPCAT (SEQ ID NO:4), 18 different single Motif I mutations were then combined with one of 16 preferred single Motif II mutations, resulting in the generation of 167 double mutants (i.e., wherein the LPCAT comprises both a single mutation within Motif I and a single mutation within Motif II). These double mutants were transformed into *Yarrowia lipolytica* strain Y8406U2, and then the lipid profiles of the double mutants were compared to that of the parent YILPCAT.

[0144] Again, the effect of each double mutation on the LPCAT activity of the resulting mutant YILPCAT protein was screened, based on EPA % TFAs and % delta-9 conversion efficiency. Most of the 167 YILPCAT mutants functioned with approximately equal or improved activity when compared to YILPCAT. More specifically, 106 of the double mutants exhibited equivalent or improved EPA % TFAs and equivalent or improved % Conv., 15 of the double mutants had an equivalent or improved EPA % TFAs when compared to the control, while an additional 6 of the double mutants were determined to have an equivalent or improved % Conv. when compared to the control.

[0145] Twenty-five (25) of these double mutants were then subjected to flask assays for a detailed analysis of the total lipid content and composition. Seventeen (17) of these double mutants were observed to have equivalent or improved EPA % TFAs and equivalent or improved % Conv., while the remaining 8 had equivalent or improved % Conv. Furthermore, 22 of these 25 mutants were demonstrated to have improved EPA productivity ["EPA % DCW"] when compared to the control strain that was not expressing a mutant YILPCAT comprising a single mutation within Motif I and a single mutation within Motif II.

[0146] Thus, disclosed herein is the amino acid sequence of a mutant YILPCAT polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:42, wherein SEQ ID NO:42 differs from SEQ ID NO:4 (YILPCAT) and wherein said difference is any one of the pairs of mutations set forth in Table 4 (e.g., an L134A mutation in Motif I may be combined with either a T382I mutation, an L388G mutation, an F390G mutation or an F390T mutation in Motif II, thereby generating mutants L134A_T382I, L134A_L388G, L134A_F390G and L134A_F390T).

Table 4. YILPCAT Double Mutations Demonstrating Equivalent or Improved EPA % TFAs and/or Equivalent or Improved % Delta-9 Conversion

Amino Acid Mutation in Motif I	Amino Acid Mutation in Motif II
L134A	T382I[b], L388G, F390G[a], F390T
L134G	L388G[a], F390G[a], F390T[a]
M136S	F378Y, T382I, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C, T389S
M136V	T382P, T382Y, P384A, L388Y, T389A, T389C, T389S
K137H	T382I[a], P384G, L388G[b], L388T, F390G[a], F390S, F390T
K137N	F378Y, T382P, R383M, P384G, L388G, L388T, T389A, T389C[b], T389S, F390G[b], F390S, F390T
S140H	T382I[b], P384G, L388G[b], L388T, F390G, F390S
S140W	T382I, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C, T389S[a]
F141M	F378Y, T382P[b], T382Y, R383M, P384A, T389A[a], T389C
F141W	F378Y, T382I[b], T382P, T382Y, R383M, P384A, L388Y[b], T389A, T389C, T389S
N144A	T382I[a], P384G, L388G, L388T, F390G, F390S, F390T
N144T	F378Y, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C, T389S
V145M	F378Y[b], T382Y[b], T382I, R383M, T389A, T389C
V145W	F378Y[b], T382I, T389A[a], T389S[a]
D147H	T382I[b], L388G, L388T, F390S, F390T[a]
D147Q	T382I, L388G[a], L388T[a], F390S
G148A	F378Y, T382I, T382Y, R383M, P384A[b], P384G, L388G, L388Y, T389A, T389C, F390S, F390T
G148N	T382I, P384G[a], L388T, F390G, F390S
Notes: Pairs ot mutations comprising a first mutation in Motif I and a second mutation in Motif II lacking a superscript (a or b) resulted in equivalent or improved EPA % TFAs and equivalent or improved % Conv.	

[a] Indicates a pair of mutations comprising a first mutation in Motif I and a second mutation in Motif II that resulted in equivalent or improved EPA % TFAs (but not equivalent or improved % Conv.).
[b] Indicates a pair of mutations comprising a first mutation in Motif I and a second mutation in Motif II that resulted in equivalent or improved % Conv. (but not equivalent or improved EPA % TFAs).

[0147] Based on the above, it will be understood by one of skill in the art that a variety of other double mutations could be generated by combining alternate single mutations within Motif I and single mutations within Motif II, wherein the single mutations are preferably selected from those that existed within the 14 YILPCAT mutants found to exhibit equivalent or improved EPA % TFAs with respect to the control or from those that existed within the 12 YILPCAT mutants found to exhibit equivalent or improved % Conv. when compared to the control. More preferably, the single mutations are those that existed within the 56 YILPCAT mutants found to exhibit equivalent or improved EPA % TFAs and equivalent or improved % Conv.

[0148] Also disclosed herein is a mutant LPCAT polypeptide encoded by the isolated polynucleotide comprising a sequence selected from the group consisting of: SEQ ID NOs:79, 81, 83, 85, 87, 89, 91 and 93.

[0149] Although certain combinations of LPCAT amino acid mutations are disclosed herein, one of skill in the art would readily recognize that other combinations of the Motif I and Motif II mutations disclosed herein may be combined as well. Accordingly, one or more of the disclosed Motif I mutations may be used in combination with one or more of the disclosed Motif II mutations in preparing a polynucleotide encoding a mutant LPCAT polypeptide.

[0150] The mutant polypeptides described herein (i.e., having at least LPCAT activity) are useful for over-expression along with over-expression of a polypeptide having PDAT activity in a recombinant microbial cell for the improved production of at least one long-chain ["LC"] polyunsaturated fatty acid, wherein over-expression of PDAT and a mutant LPCAT results in an increase in the at least one long-chain polyunsaturated fatty acid, measured as a wt. % TFAs, when compared to a control cell. It should also be noted that these results are also achieved upon over-expression of mutant LPCAT polypeptides described herein without over-expression of PDAT.

[0151] Specifically, disclosed herein is a recombinant cell comprising any one of the isolated polynucleotides described herein, encoding a non-naturally occurring mutant polypeptide having LPCAT activity, wherein said recombinant cell further comprises a PUFA biosynthetic pathway capable of producing at least one long-chain polyunsaturated fatty acid, and wherein the isolated polynucleotide is over-expressed, and wherein the recombinant cell comprises at least one of the following:

1. (a) an amount of at least one long-chain polyunsaturated fatty acid measured as a weight percent of total fatty acids that is at least the same as or greater than the amount produced by a control cell, or
2. (b) a C_{18} to C_{20} elongation conversion efficiency (e.g., delta-9 elongase conversion efficiency or delta-6 elongase conversion efficiency) that is at least the same as or greater than the conversion efficiency of a control cell.

[0152] With respect to over-expressing a mutant LPCAT (containing a mutation in Motif I and/or Motif II) in a recombinant cell, over-expression of a mutant LPCAT can be achieved, for example, by introducing a polynucleotide encoding mutant LPCAT (i.e., transgene) to cells. Preferably, such a polynucleotide is operably linked to a regulatory sequence such as a promoter that allows gene expression in the cells (e.g., microbial cells) modified to contain the polynucleotides. Over-expression of mutant LPCAT is with respect to the expression of LPCAT in a control cell.

[0153] An increase in the amount of the at least one long-chain PUFA (e.g., EPA) measured as a weight percent of total fatty acids ["wt. % TFAs"] of the recombinant cell over-expressing a mutant LPCAT (containing a mutation in Motif I and/or Motif II) may be at least about 1 %, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11 %, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% over the amount of the at least one long-chain PUFA measured as a weight percent of total fatty acids of a control cell.

[0154] An increase in the C_{18} to C_{20} elongation conversion efficiency, delta-9 elongase conversion efficiency, and/or delta-6 elongase conversion efficiency of the recombinant cell over-expressing a mutant LPCAT (containing a mutation in Motif I and/or Motif II) may be at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% over the C_{18} to C_{20} elongation conversion efficiency, delta-9 elongase conversion efficiency, and/or delta-6 elongase conversion efficiency, respectively, of a control cell.

[0155] Total lipid content (TFAs % DCW) may be increased in the recombinant cell over-expressing mutant LPCAT. The increase in the total lipid content of the recombinant cell may be at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11 %, or 12% over the total lipid content of a control cell. The increase in total lipid content can coincide with an increase in EPA % TFAs.

[0156] With respect to over-expressing a mutant LPCAT, a control cell, corresponding control cell, or suitable control cell may be a

wild type or recombinant cell that corresponds to the recombinant cell, but does not comprise the over-expressed mutant LPCAT polypeptide. For example, the control cell does not over-express a mutant LPCAT polypeptide by virtue of not comprising recombinant polynucleotide sequences encoding mutant LPCAT. Also for example, the control cell does not over-express mutant LPCAT polypeptides by virtue of comprising, but not expressing, a recombinant polynucleotide sequence encoding mutant LPCAT. The control cell may be the recombinant cell as it existed before it was modified to over-express a mutant LPCAT polypeptide (i.e., a parent cell), or may be a recombinant cell that has been modified to contain a recombinant polynucleotide encoding mutant LPCAT, but does not over-express the mutant LPCAT polypeptide (e.g., a cell prepared in parallel with the recombinant cell that over-expresses a mutant LPCAT).

[0157] One of ordinary skill in the art is aware of standard resource materials that describe: 1) specific conditions and procedures for construction, manipulation and isolation of macromolecules, such as DNA molecules, plasmids, etc.; 2) generation of recombinant DNA fragments and recombinant expression constructs; and, 3) screening and isolating of clones. See, Maniatis, Silhavy, and Ausubel, as cited above.

[0158] In general, the choice of sequences included in a recombinant expression construct depends on the desired expression products, the nature of the host cell and the proposed means of separating transformed cells versus non-transformed cells. Typically, a vector contains at least one expression cassette, a selectable marker and sequences allowing autonomous replication or chromosomal integration. Suitable expression cassettes typically comprise a promoter, the coding sequence of a selected gene (e.g., encoding a polypeptide having at least LPCAT or PDAT activity), and a terminator (i.e., a chimeric gene). Preferably, both control regions are derived from genes from the transformed host cell.

[0159] Virtually any promoter (i.e., native, synthetic, or chimeric) capable of directing expression of an ORF encoding a polypeptide of the invention herein will be suitable, although transcriptional and translational regions from the host species are particularly useful. Expression in a host cell can occur in an induced or constitutive fashion. Induced expression occurs by inducing the activity of a regulatable promoter operably linked to the LPCAT and/or PDAT gene(s) of interest, while constitutive expression occurs by the use of a constitutive promoter operably linked to the gene(s) of interest.

[0160] A terminator can be derived from the 3' region of a gene from which the promoter was obtained or from a different gene. A large number of termination regions are known and function satisfactorily in a variety of hosts when utilized in both the same and different genera and species from which they were derived. The terminator usually is selected more as a matter of convenience rather than because of any particular property. Preferably, the terminator is derived from a yeast gene. The terminator can also be synthetic, as one of skill in the art can utilize available information to design and synthesize a terminator. A terminator may be unnecessary, but it is highly preferred.

[0161] Many specialized expression vectors have been created to obtain a high expression rate. Such vectors are made by adjusting certain properties that govern transcription, RNA stability, translation, protein stability and location, and secretion from the host cell. These properties include: the nature of the relevant transcriptional promoter and terminator sequences; the number of copies of the cloned gene (wherein additional copies may be cloned within a single expression construct and/or additional copies may be introduced into the host cell by increasing the plasmid copy number or by multiple integration of the cloned gene into the genome); whether the gene is plasmid-borne or integrated into the host cell genome; the final cellular location of the synthesized protein; the efficiency of translation and correct folding of the protein in the host organism; the intrinsic stability of the mRNA and protein of the cloned gene within the host cell; and, the codon usage within the cloned gene, such that its frequency approaches the frequency of preferred codon usage of the host cell.

[0162] Once a DNA cassette (e.g., comprising a chimeric gene comprising a promoter, an ORF encoding a polypeptide having LPCAT activity or PDAT activity, and a terminator) suitable for expression in an appropriate cell has been obtained, it is placed in a plasmid vector capable of autonomous replication in the host cell or it is directly integrated into the genome of the host cell. Integration of expression cassettes can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

[0163] Constructs comprising a chimeric gene(s) of interest may be introduced into e.g., oleaginous yeast by any standard technique. These techniques include transformation (e.g., lithium acetate transformation [Methods in Enzymology, 194:186-187 (1991)]), biolistic impact, electroporation, microinjection, or any other method that introduces the gene(s) of interest into the host cell. More specific teachings applicable for *Y. lipolytica* include U.S. Pat. No. 4,880,741 and U.S. Pat. No. 5,071,764 and Chen et al. (Appl. Microbiol. Biotechnol., 48(2):232-235 (1997)). Integration of a linear DNA fragment into the genome of the host is favored in transformation of *Y. lipolytica* host cells. Integration into multiple locations within the genome can be particularly useful when high level expression of genes are desired. Preferred loci include those taught in U.S. Pat. Appl. Publ. No. 2009-0093543-A1.

[0164] The transformed host cell can be identified by selection for a marker contained on the introduced construct. Alternatively, a

separate marker construct may be co-transformed with the desired construct, as many transformation techniques introduce many DNA molecules into host cells.

[0165] Stability of an integrated DNA fragment in a microbial host cell is often dependent on the individual transformants, the recipient strain and the targeting platform used. Thus, multiple transformants of a particular recombinant microbial host should be screened in order to obtain a strain displaying the desired expression level and pattern. Southern analysis of DNA blots (Southern, J. Mol. Biol., 98:503 (1975)), northern analysis of mRNA expression (Kroczek, J. Chromatogr. Biomed. Appl., 618(1-2):133-145 (1993)), western analysis of protein expression, phenotypic analysis or GC analysis are suitable screening methods.

[0166] Disclosed herein are recombinant constructs that comprise the isolated polynucleotides of the invention. For example, a recombinant construct may comprise an isolated polynucleotide encoding a non-naturally occurring mutant polypeptide having LPCAT activity, wherein the mutant polypeptide comprises at least one mutant membrane MBOAT protein motif, operably linked to at least one regulatory sequence.

[0167] Disclosed herein are recombinant cells that comprise the recombinant constructs of the invention. The recombinant cells described herein all comprise a PUFA biosynthetic pathway capable of producing at least one LC polyunsaturated fatty acid. Preferably, the long-chain polyunsaturated fatty acid is selected from the group consisting of: eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, omega-6 docosapentaenoic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid, omega-3 docosapentaenoic acid and docosahexaenoic acid.

[0168] The metabolic process wherein oleic acid is converted to LC-PUFAs involves elongation of the carbon chain through the addition of carbon atoms and desaturation of the molecule through the addition of double bonds. This requires a series of special desaturation and elongation enzymes present in the endoplasmic reticulum membrane. However, as seen in FIG. 2 and as described below, multiple alternate pathways exist for LC-PUFA production.

[0169] Specifically, FIG. 2 depicts the pathways described below. All pathways require the initial conversion of oleic acid to linoleic acid ["LA"], the first of the omega-6 fatty acids, by a delta-12 desaturase. Then, using the "delta-9 elongase/ delta-8 desaturase pathway" and LA as substrate, long-chain omega-6 fatty acids are formed as follows: 1) LA is converted to eicosadienoic acid ["EDA"] by a delta-9 elongase; 2) EDA is converted to dihomo-gamma-linolenic acid ["DGLA"] by a delta-8 desaturase; 3) DGLA is converted to arachidonic acid ["ARA"] by a delta-5 desaturase; 4) ARA is converted to docosatetraenoic acid ["DTA"] by a $C_{20/22}$ elongase; and 5) DTA is converted to docosapentaenoic acid ["DPAn-6"] by a delta-4 desaturase.

[0170] The "delta-9 elongase/delta-8 desaturase pathway" can also use alpha-linolenic acid ["ALA"] as substrate to produce long-chain omega-3 fatty acids as follows: 1) LA is converted to ALA by a delta-15 desaturase; 2) ALA is converted to eicosatrienoic acid ["ETrA"] by a delta-9 elongase; 3) ETrA is converted to eicosatetraenoic acid ["ETA"] by a delta-8 desaturase; 4) ETA is converted to eicosapentaenoic acid ["EPA"] by a delta-5 desaturase; 5) EPA is converted to docosapentaenoic acid ["DPA"] by a $C_{20/22}$ elongase; and 6) DPA is converted to docosahexaenoic acid ["DHA"] by a delta-4 desaturase. Optionally, omega-6 fatty acids may be converted to omega-3 fatty acids. For example, ETA and EPA are produced from DGLA and ARA, respectively, by delta-17 desaturase activity.

[0171] Alternate pathways for the biosynthesis of omega-3/omega-6 fatty acids utilize a delta-6 desaturase and $C_{18/20}$ elongase, that is, the "delta-6 desaturase/delta-6 elongase pathway". More specifically, LA and ALA may be converted to GLA and stearidonic acid ["STA"], respectively, by a delta-6 desaturase; then, a $C_{18/20}$ elongase converts GLA to DGLA and/or STA to ETA.

[0172] A LC-PUFA-producing recombinant cell will possess at least one of the biosynthetic pathways described above, whether this pathway is native to the cell or is genetically engineered. Preferably, the recombinant cell will be capable of producing at least about 2-5% LC-PUFAs in the total lipids of the recombinant cell, more preferably at least about 5-15% LC-PUFAs in the total lipids, more preferably at least about 15-35% LC-PUFAs in the total lipids, more preferably at least about 35-50% LC-PUFAs in the total lipids, more preferably at least about 50-65% LC-PUFAs in the total lipids and most preferably at least about 65-75% LC-PUFAs in the total lipids. The structural form of the LC-PUFAs is not limiting; thus, for example, the EPA or DHA may exist in the total lipids as free fatty acids or in esterified forms such as acylglycerols, phospholipids, sulfolipids or glycolipids.

[0173] An "LC polyunsaturated fatty acid" refers to the PUFA that the PUFA biosynthetic pathway is designed to produce. Thus, for example, in the present examples, a *Yarrowia lipolytica* strain engineered to express a PUFA biosynthetic pathway comprising delta-12 desaturase, delta-9 elongase, delta-8 desaturase, delta-5 desaturase and delta-17 desaturase genes produced a variety of fatty acids in the lipids including palmitate, palmitoleic acid, stearic acid, oleic acid, LA, ALA, EDA, DGLA, ARA, ETrA, ETA, EPA. However, since the strain was designed to primarily produce EPA as the product of the PUFA biosynthetic pathway, this fatty acid should be considered as the LC polyunsaturated product fatty acid.

[0174] A variety of eukaryotes such as plants, fungi and microbial organisms, including yeast, algae, stramenopiles, oomycetes and euglenoids can be used herein to produce (or can be engineered to produce) LC-PUFAs. These may include cells that grow on a

variety of feedstocks, including simple or complex carbohydrates, fatty acids, organic acids, oils, glycerols and alcohols, and/or hydrocarbons over a wide range of temperature and pH values. Thus, any of these organisms are suitable host cells for transformation with the polynucleotides of the invention.

[0175] Preferred microbes are oleaginous organisms. These oleaginous organisms are naturally capable of oil synthesis and accumulation, wherein the total oil content can comprise greater than about 25% of the dry cell weight, more preferably greater than about 30% of the dry cell weight, and most preferably greater than about 40% of the dry cell weight. Various bacteria, algae, euglenoids, moss, fungi, yeast and stramenopiles are naturally classified as oleaginous. Within this broad group of microbes, of particular interest are those organisms that naturally produce omega-3/omega-6 fatty acids. For example, ARA, EPA and/or DHA is produced by *Cyclotella* sp., *Crypthecodinium* sp., *Mortierella* sp., *Nitzschia* sp., *Pythium, Thraustochytrium* sp. and *Schizochytrium* sp. Thus, for example, transformation of *Mortierella alpina,* which is commercially used for production of ARA, with any of the present LPCAT genes (optionally with co-expression of PDAT) under the control of inducible or regulated promoters could yield a transformant organism capable of synthesizing increased quantities of ARA. The method of transformation of *M. alpina* is described by Mackenzie et al. (Appl. Environ. Microbiol., 66:4655 (2000)). Similarly, methods for transformation of Thraustochytriales microorganisms (e.g., *Thraustochytrium, Schizochytrium*) are disclosed in U.S. Pat. No. 7,001,772. In alternate embodiments, a non-oleaginous organism can be genetically modified to become oleaginous, e.g., yeast such as *Saccharomyces cerevisiae* (U.S. Pat. Appl. Publ. No. 2007/0015237-A1).

[0176] In more preferred embodiments, the microbial cells are oleaginous yeast. Genera typically identified as oleaginous yeast include, but are not limited to: *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon* and *Lipomyces.* More specifically, illustrative oil-synthesizing yeast include: *Rhodosporidium toruloides, Lipomyces starkeyii, L. lipoferus, Candida revkaufi, C. pulcherrima, C. tropicalis, C. utilis, Trichosporon pullans, T. cutaneum, Rhodotorula glutinus, R. graminis* and *Yarrowia lipolytica* (formerly classified as *Candida lipolytica*). Most preferred is the oleaginous yeast *Yarrowia lipolytica;* and in a further embodiment, most preferred are the *Y. lipolytica* strains designated as ATCC #76982, ATCC #20362, ATCC #8862, ATCC #18944 and/or LGAM S(7)1 (Papanikolaou S., and Aggelis G., Bioresour. Technol., 82(1):43-9 (2002)).

[0177] Specific teachings applicable for engineering ARA, EPA and DHA production in *Y. lipolytica* are provided in U.S. Pat. 7,588,931, U.S. Pat. 7,932,077, U.S. Pat. Appl. Publications No. 2009-0993543-A1, No. 2010-0317072-A1 and No. 2012-0052537-A1, and U.S. Pat. 7,550,286, respectively. These references also describe the preferred method of expressing genes in *Yarrowia lipolytica* by integration of linear DNA fragments into the genome of the host, preferred promoters, termination regions, integration loci and disruptions, and preferred selection methods when using this particular host species.

[0178] Similarly, a variety of plants may produce (or be engineered to produce) at least one LC polyunsaturated fatty acid (see, e.g., PCT Publ. No. WO 1998/46764, U.S. Pat. Appl. Publ. No. 2004-0172682-A1) and thus are suitable host cells for transformation with the polynucleotides described herein. For example, U.S. Pat. Appl. Publ. No. 2008-0254191-A1 provides a detailed discussion concerning oleaginous plants, which are commonly referred to as "oilseed" plants (which include, e.g., soybean [*Glycine* and *Soja sp.*], rapeseed [*Brassica sp.],* sunflower [*Helianthus sp.*], maize, cotton, flax [*Linum sp.*] and safflower [*Carthamus sp.*]), as well as means to engineer suitable recombinant constructs for these species and enable transformations and regeneration of the transformed plant tissue and cells.

[0179] The transformed recombinant cell is grown under conditions that optimize expression of chimeric genes of the invention and produce the greatest and the most economical yield of the LC polyunsaturated fatty acid(s). In general, media conditions may be optimized by modifying the type and amount of carbon source, the type and amount of nitrogen source, the carbon-to-nitrogen ratio, the amount of different mineral ions, the oxygen level, growth temperature, pH, length of the biomass production phase, length of the oil accumulation phase and the time and method of cell harvest.

[0180] *Yarrowia lipolytica* is generally grown in a complex media such as yeast extract-peptone-dextrose broth ["YPD"] or a defined minimal media that lacks a component necessary for growth and thereby forces selection of the desired expression cassettes (e.g., Yeast Nitrogen Base (DIFCO Laboratories, Detroit, MI)).

[0181] Fermentation media for the methods and host cells described herein must contain a suitable carbon source, such as are taught in U.S. Pat. No. 7,238,482 and U.S. Pat. Appl. Publ. No. 2011-0059204-A1. Although it is contemplated that the source of carbon utilized may encompass a wide variety of carbon-containing sources, preferred carbon sources are sugars, glycerol and/or fatty acids. Most preferred is glucose, sucrose, invert sucrose, fructose and/or fatty acids containing between 10-22 carbons. For example, the fermentable carbon source can be selected from the group consisting of invert sucrose, glucose, fructose and combinations of these, provided that glucose is used in combination with invert sucrose and/or fructose.

[0182] Nitrogen may be supplied from an inorganic (e.g., $(NH_4)_2SO_4$) or organic (e.g., urea or glutamate) source. In addition to appropriate carbon and nitrogen sources, the fermentation media must also contain suitable minerals, salts, cofactors, buffers, vitamins and/or other components known to those skilled in the art suitable for the growth of the host cells and the promotion of the enzymatic pathways for LC polyunsaturated fatty acid production. Particular attention is given to several metal ions, such as Fe^{+2},

Cu^{+2}, Mn^{+2}, Co^{+2}, Zn^{+2} and Mg^{+2} that promote synthesis of lipids and PUFAs (Nakahara, T. et al., Ind. Appl. Single Cell Oils, D. J. Kyle and R. Colin, eds. pp 61-97 (1992)).

[0183] Preferred growth media for the methods and host cells described herein are common commercially prepared media, such as Yeast Nitrogen Base (DIFCO Laboratories, Detroit, MI). Other defined or synthetic growth media may also be used and the appropriate medium for growth of *Yarrowia lipolytica* will be known by one skilled in the art of microbiology or fermentation science. A suitable pH range for the fermentation is typically between about pH 4.0 to pH 8.0, wherein pH 5.5 to pH 7.5 is preferred as the range for the initial growth conditions. The fermentation may be conducted under aerobic or anaerobic conditions, wherein microaerobic conditions are preferred.

[0184] Typically, accumulation of high levels of PUFAs in oleaginous yeast cells requires a two-stage process, since the metabolic state must be "balanced" between growth and synthesis/storage of fats. Thus, most preferably, a two-stage fermentation process is necessary for the production of LC polyunsaturated fatty acid(s) in *Yarrowia lipolytica.* This approach is described in U.S. Pat. No. 7,238,482, as are various suitable fermentation process designs (i.e., batch, fed-batch and continuous) and considerations during growth.

[0185] Thus, in one aspect, the present invention is directed toward a method for improving the production of at least one LC polyunsaturated fatty acid having a chain length of C20 or greater, said method comprising:

1. (a) growing the recombinant microbial cell of the invention in the presence of a fermentable carbon source; and
2. (b) optionally recovering the LC polyunsaturated fatty acid.

Preferably, the recombinant microbial cell grown in this method is an oleaginous yeast such as one of the genus *Yarrowia* (e.g., *Y. lipolytica*). The LC PUFA produced by the method is preferably selected from the group consisting of: eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, omega-6 docosapentaenoic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid, omega-3 docosapentaenoic acid and docosahexaenoic acid.

EXAMPLES

[0186] The present invention is further described in the following Examples, which illustrate reductions to practice of the invention but do not completely define all of its possible variations.

GENERAL METHODS

[0187] Standard recombinant DNA and molecular cloning techniques used in the Examples are well known in the art and are described by: 1) Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989); 2) T. J. Silhavy, M. L. Bennan, and L. W. Enquist, Experiments with Gene Fusions; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1984); and, 3) Ausubel, F. M. et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience, Hoboken, NJ (1987).

[0188] Materials and methods suitable for the maintenance and growth of microbial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in Manual of Methods for General Bacteriology (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, Eds, American Society for Microbiology: Washington, D.C. (1994)); or by Thomas D. Brock in Biotechnology: A Textbook of Industrial Microbiology, 2nd ed., Sinauer Associates: Sunderland, MA (1989). All reagents, restriction enzymes and materials used for the growth and maintenance of microbial cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), New England Biolabs, Inc. (Beverly, MA), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO), unless otherwise specified. *E. coli* strains were typically grown at 37 °C on Luria Bertani ["LB"] plates.

[0189] General molecular cloning was performed according to standard methods (Sambrook et al., above). DNA sequence was generated on an ABI Automatic sequencer using dye terminator technology using a combination of vector and insert-specific primers. Sequence editing was performed in Sequencher (Gene Codes Corporation, Ann Arbor, MI).

[0190] *Yarrowia lipolytica* strain ATCC #20362 was purchased from the American Type Culture Collection (Manassas, VA). *Y. lipolytica* strains were routinely grown at 28-30 °C in several media (e.g., Basic Minimal Media ["MM"], Minimal Media + 5-Fluoroorotic Acid ["MM + 5-FOA"], High Glucose Media ["HGM"] and Fermentation medium ["FM"]), as described in U.S. Pat. Appl. Publ. No. 2009-0093543-A1.

[0191] Transformation of *Y. lipolytica* was performed as described in U.S. Pat. Appl. Publ. No. 2009-0093543-A1.

[0192] For fatty acid ["FA"] analysis, cells were collected by centrifugation and lipids were extracted as described by Bligh and Dyer (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 and Nishida, 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.

[0193] 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.

[0194] Alternately, a modification of the base-catalyzed 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 half-way up the tube and inserted into a fresh 2-mL round bottom Eppendorf tube (Cat. No. 22 36 335-2).

[0195] 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 (above) in toluene was added and 500 µl of freshly made 1% sodium methoxide in methanol solution. The sample pellet was firmly broken up 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 2 x 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.

[0196] 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 ["µg 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.

[0197] The lipid profile, summarizing the amount of each individual fatty acid as a weight percent of TFAs (i.e., FA % TFAs), was determined by dividing the individual FAME peak area by the sum of all FAME peak areas and multiplying by 100.

[0198] For quantitating the amount of an individual fatty acid or the total fatty acids as a weight percent of the dry cell weight ["% DCW"], cells from 10 mL of the culture were collected by centrifugation, washed once with 10 mL water and collected by centrifugation again. Cells were resuspended in 1-2 mL water, poured into a pre-weighed aluminum weighing pan, and rinsed with 1-2 mL water that was also added to the same weighing pan. The pan was placed under vacuum at 80 °C overnight. The pan was weighed and the DCW calculated by subtracting the weight of the empty pan. Determination of the fatty acid as a % DCW can then be calculated based on either µg FAME or µg FA as a fraction of the µg DCW (for example, FAME % DCW was calculated as µg FAME/µg DCW*100).

[0199] 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 shaking 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 shaking incubator at 250 rpm and at 30 °C, a 1 mL aliquot was used for fatty acid analysis and 10 mL dried for dry cell weight determination.

EXAMPLE 1

Isolation of *Yarrowia lipolytica* LPCAT

[0200] U.S. Pat. Appl. Publ. No. 2010-0317882-A1 describes the identification of a *Y. lipolytica* homolog to the *Saccharomyces cerevisiae* Ale1 (i.e., "ScAle1"; SEQ ID NO:2; GenBank Accession No. NP_014818; U.S. Pat. No. 7,732,155; Intl. Appl. Publ. No. WO 2009/001315). This homolog, designated therein as either YlAle1 or YILPCAT (SEQ ID NO:4) and corresponding to ORF YALI0F19514p (GenBank Accession No. XP_505624; Intl. Appl. Publ. No. WO 2009/001315) was found to be 45% identical to ScAle1.

[0201] YILPCAT was analyzed to determine the presence or absence of non-plant motifs present in Ale1 homologs, as identified in U.S. Pat. No. 7,732,155 and U.S. Pat. Appl. Publ. No. 2008-0145867-A1. Specifically, these motifs are SEQ ID NOs:8-15 (Table 2). The His residue in SEQ ID NO:11 (SAxWHG-X2-PGY-X2-[T/F]-F) may be an active site residue within the protein, given studies of other LPCATs (Lee et al., 2008, Mol. Biol. Cell 19:1174-1184). It was determined that YILPCAT comprises at least the motifs SEQ ID NOs:8-11. It is hypothesized herein that these conserved motifs are likely involved in catalysis.

[0202] Overexpression of YILPLAT in a strain of *Y. lipolytica* that had been engineered to produce EPA resulted in a significant reduction of the concentration of LA (18:2) as a weight % of TFAs ["LA % TFAs"], an increase in the concentration of EPA as a weight % of TFAs ["EPA % TFAs"], and an increase in the conversion efficiency of delta-9 elongase (U.S. Pat. Appl. Publ. No. 2010-0317882-A1).

EXAMPLE 2

Co-Expression of PDAT with LPCAT or LPAAT in *Yarrowia lipolytica*

[0203] The present Example describes overexpression of a *Y. lipolytica* PDAT (phospholipid:diacylglycerol acyltransferase [EC 2.3.1.158]) with either a *Y. lipolytica* LPCAT (acyl-CoA:lysophosphatidylcholine acyltransferase [EC 2.3.1.23]) or a *Y. lipolytica* LPAAT (acyl CoA:lysophosphatidic acid acyltransferase [EC 2.3.1.51]) in a *Y. lipolytica* strain that had been engineered to produce a high level of lipids containing eicosapentaenoic acid ["EPA"]. Compared to *Yarrowia* transformants co-expressing PDAT and LPAAT, transformants co-expressing PDAT and LPCAT produced an increased amount of EPA, measured as a weight percent of total fatty acids (EPA % TFAs). Furthermore, PDAT and LPCAT co-expression resulted in an increased C_{18} to C_{20} elongation conversion efficiency, measured as increased delta-9 elongase percent conversion efficiency, and an increased amount of total fatty acids, measured as a weight percent of the dry cell weight (TFAs % DCW).

Construction of Vectors for Overexpression of PDAT with LPAAT or LPCAT

[0204] To test if the enzymatic activities of PDAT and LPCAT could function synergistically to improve oil and EPA production in *Yarrowia,* the effects of co-expressing PDAT with LPAAT were compared to the effects of co-expressing PDAT with LPCAT.

[0205] Plasmids pY196 (FIG. 3A, SEQ ID NO:43) and pY301 (FIG. 3B, SEQ ID NO:44) were constructed to co-express these enzyme pairs in *Y. lipolytica.* As listed in Tables 5 and 6, respectively, both of these plasmids contained a chimeric YAT1::YlPDAT::Pex16 gene for expressing wild type *Y. lipolytica* PDAT (SEQ ID NO:32). pY196 also contained a chimeric FBAINm::YILPAAT1::Lip1 gene for expressing wild type *Y. lipolytica* LPAAT1 (SEQ ID NO:23), while pY301 also contained a chimeric YAT1::YILPCAT::Lip1 gene for expressing wild type *Y. lipolytica* LPCAT (SEQ ID NO:4).

Table 5. Components of Plasmid pY196 (SEQ ID NO:43)

RE Sites and Nucleotides within SEQ ID NO:43	Description of Fragment and Chimeric Gene Components
*Sph*I/*Avr*II	Fragment of *Y. lipolytica URA3* gene (GenBank Accession No. AJ306421; labeled as "U3 repeat" in Figure 3A)
1-875	
*Avr*II/*Pac*I	• ColE1 plasmid origin of replication
875-3078	• Ampicillin-resistance gene
*Pac*I/*Sal*I 3078-4570	*Y. lipolytica URA3* gene (GenBank Accession No. AJ306421)
*Sal*I/*Pme*I	YAT1::YlPDAT::PEX16, comprising:
4570-7624	• YAT1: *Y. lipolytica* YAT1 promoter (U.S. Pat. Appl. Publ. No. 2010/0068789);
	• YlPDAT: *Y. lipolytica* phospholipid:diacylglycerol acyltransferase gene (SEQ ID NO:32; U.S. Pat. No. 7,901,928; GenBank Accession No. XM_504038);
	• PEX16 terminator sequence from *Yarrowia PEX16* gene (GenBank Accession No. YLU75433)
*Pme*I/*Swa*I 7624-8919	Kanamycin-resistance gene from plasmid pBHR1 (GenBank Accession No.

RE Sites and Nucleotides within SEQ ID NO:43	Description of Fragment and Chimeric Gene Components
	Y14439)
*Swa*I/*Sph*I	FBAINm::YILPAAT1::Lip1 (complementary), comprising:
8919-1	• FBAINm: *Y. lipolytica* FBAINm promoter (U.S. Pat. No. 7,202,356);
	• YILPAAT1: *Y. lipolytica* acyl-CoA:lysophosphatidic acid acyltransferase gene (SEQ ID NO:23; U.S. Pat. No. 7,189,559; GenBank Accession No. XP_504127);
	• Lip1: terminator sequence from *Yarrowia Lip1* gene (GenBank Accession No. Z50020)

Table 6. Components of Plasmid pY301 (SEQ ID NO:44)

RE Sites and Nucleotides within SEQ ID NO:44	Description of Fragment and Chimeric Gene Components
*Sph*I/*Avr*II	Fragment of *Y. lipolytica URA3* gene (GenBank Accession No. AJ306421; labeled as "U3 repeat" in Figure)
1-875	
*Avr*II/*Pac*I	ColE1 plasmid origin of replication
875-2079	
*Pac*I/*Sal*I	*Y. lipolytica URA3* gene (GenBank Accession No. AJ306421)
2079-3571	
*Sal*I/*Pme*I	YAT1::YIPDAT::PEX16 (as described in Table 5 for pY196)
3571-6625	
*Pme*I/*Swa*I	Kanamycin-resistance gene from plasmid pBHR1 (GenBank Accession No. Y14439)
6625-7920	
*Swa*I/*Sph*I	YAT1::YILPCAT::Lip1 (complementary), comprising:
7920-1	• YAT1: *Y. lipolytica* YAT1 promoter (U.S. Pat. Appl. Publ. No. 2010-0068789-A1);
	• YILPCAT: *Y. lipolytica* lysophosphatidylcholine acyltransferase gene (SEQ ID NO:4; U.S. Pat. Appl. Publ. No. 2010/0317882);
	• Lip1: terminator sequence from *Yarrowia Lip1* gene (GenBank Accession No. Z50020)

Lipid Production in *Y. lipolytics* Strain Z5567U19 Transformed with pY196 or pY301

[0206] Plasmids pY196 and pY301 were digested with *Pme*I and *Swa*I. The larger fragment in each digestion was agarose-purified away from the kanamycin-resistance gene fragment and used to transform *Yarrowia* strain Z5567U19 by chromosomal integration. Z5567U19 is a *Ura*$^-$ strain of Z5567 and produces an increased amount of lipids containing long-chain polyunsaturated fatty acids. Details regarding the development of strains Z5567 and Z5567U19 are provided in U.S. Pat. Appl. Publ. No. 2012-0052537 A1. A control transformation was also performed in which no plasmid DNA was included.

[0207] The transformed cells were plated onto MM plates and maintained at 30 °C for 5 days (MM comprises per liter: 20 g glucose, 1.7 g yeast nitrogen base without amino acids, 1.0 g proline, pH 6.1 (do not need to adjust)). Eleven colonies for each experimental transformation (i.e., either PDAT+LPCAT [pY301] or PDAT+LPAAT [pY196]) were then re-streaked onto MM plates and subsequently analyzed for lipid content.

[0208] Table 7 summarizes the total dry cell weight ["DCW"], TFAs % DCW, the concentration of EPA as a weight percent of TFAs ["EPA % TFAs"], EPA % DCW, and the total delta-9 elongase percent conversion efficiency ["d9E CE"] of LA and ALA to EPA in each transformant and the control. Calculation of d9e CE was made following the formula: (EDA + HGLA + ARA + ERA + ETA + EPA) / (C18:2 + C18:3 + EDA + HGLA + ARA + ERA + ETA + EPA) * 100.

Table 7. Lipid Analysis of pY196 and pY301 Transformants of *Yarrowia* Strain Z5667U19, by Flask Assay

Z5567U19 transformant	Transformation plasmid	DCW, (g/L)	TFAs % DCW	EPA % TFAs	EPA % DCW	d9e CE (%)
L313	Control	5.9	46.1	45	21	76
L313		5.7	48.9	46	23	77
Average		**5.8**	**47.5**	**46**	**22**	**76**
Standard deviation		0.1	2.0	0.4	1.1	0.6

Z5567U19 transformant	Transformation plasmid	DCW, (g/L)	TFAs % DCW	EPA % TFAs	EPA % DCW	d9e CE (%)
	pY196 (PDAT+LPAAT)	3.1	39.7	49	19	79
		3.2	41.9	51	21	81
L314		4.1	48.4	49	24	79
		3.7	47.0	50	23	79
		3.4	39.5	46	18	77
		5.1	42.9	46	20	77
		3.6	46.8	48	22	78
		4.3	43.7	49	22	78
		4.2	46.6	49	23	79
		3.8	45.9	49	22	78
		4.7	46.4	47	22	79
Average		**3.9**	**44.5**	**48**	**22**	**79**
Standard deviation		0.6	3.1	1.6	1.8	1.2
	pY301 (PDAT+LPCAT)	4.3	37.7	45	17	78
		4.9	48.2	51	25	83
L317		4.7	49.0	51	25	82
		4.6	48.1	51	24	82
		4.2	44.6	50	22	81
		5.5	43.6	51	22	82
		4.8	44.8	50	22	80
		4.7	46.0	49	23	81
		4.1	41.2	46	19	79
		4.3	46.5	49	23	81
		5.2	47.4	51	24	81
Average		**4.7**	**45.2**	**49**	**22**	**81**
Standard deviation		0.4	3.4	2.0	2.4	1.5

[0209] Both the pY196 and pY301 transformants had improved EPA % TFAs and d9e CE compared to the control. Specifically regarding the pY301 transformants (PDAT+LPCAT), they exhibited an average increase in EPA % TFAs and d9e CE of about 6.5% and 6.6%, respectively, over the control. Furthermore, the pY301 transformants had average DCW, TFAs % DCW, EPA % TFAs and d9E CE values that, respectively, were 20.5%, 1.6%, 2.1% and 2.5% greater than the respective average values measured for the pY196 transformants.

[0210] Differences in the lipids of certain individual transformants were also compared. Specifically, the lipid profiles of the pY196 transformant L314 and the pY301 transformant L317 were further analyzed (Table 8) in comparison to each other and the control, strain L313.

Table 8. Comparison of Lipid Production in Transformants L314 and L317

Z5567U19 transformant	DCW, (g/L)	TFAs % DCW	EPA % TFAs	EPA % DCW	d9e CE (%)
L313 control, average	5.8	47.5	45.8	21.7	76.2
L314 (pY196, PDAT+LPAAT)	4.1	48.4	49.3	23.9	78.6
L314, % change over control:	-30	1.9	7.7	9.7	3.2
L317 (pY301, PDAT+LPCAT)	4.7	49.0	51.0	25.0	81.9
L317, % change over control:	-19	3.2	11.4	15.2	7.5
L317, % change over L314:	14.6	1.2	3.4	4.6	4.2

[0211] Transformant L317 had improved TFAs % DCW, EPA % TFAs, EPA % DCW and d9e CE compared to both the control and transformant L314.

[0212] Previous attempts to enhance lipids in *Yarrowia* by other strategies have mostly yielded increased total lipid content [TFAs %

DCW], but with a decrease in the EPA concentration as a weight percent of TFAs [EPA % TFAs], or vice versa (i.e., lower TFAs % DCW with higher EPA % TFAs). In transformant L317, however, both of these factors increased with respect to the control and L314. Therefore, the concomitant overexpression of PDAT and LPCAT in transformant L317 may allow a balanced movement of EPA from acyl-CoA stores (i.e., EPA-CoA) to TAG by increasing the rate at which EPA contained in phosphatidylcholine ["PC"] is transferred to DAG while also increasing the rate at which PC is restored from lysophosphatidylcholine using EPA-CoA.

[0213] Overexpression of PDAT and LPCAT (strain L317) appears to have advantages when compared to overexpression of PDAT and LPAAT (strain L314). This may point to a greater synergy between PDAT and LPCAT than between PDAT and LPAAT in the synthesis of TAG using phospholipid-derived fatty acids. In both overexpression systems, PDAT transferred fatty acids from PC and phosphatidic acid ["PA"] stores to DAG. The higher level of lipid production observed using PDAT and LPCAT, as compared to PDAT and LPAAT, may reflect a heretofore unappreciated difference in the rate of renewal of PC and PA by LPCAT and LPAAT, respectively, as fatty acid sources for continued PDAT activity.

EXAMPLE 3

Synthesis of Plasmid pY306-N Comprising Variant YlLPCAT

[0214] The present example describes the construction of a *Yarrowia* autonomously replicating vector comprising a variant YlLPCAT sequence (plasmid pY306-N, SEQ ID NO:48). The variant YlLPCAT polynucleotide sequence, designated herein as YlLPCAT* (SEQ ID NOs:45), lacks two *Nco*I restriction enzyme sites that are present in the wild type YlLPCAT coding region. Removal of these internal *Nco*I sites facilitated subsequent cloning procedures.

[0215] As a control, the wild type YlLPCAT ORF (SEQ ID NO:3; Example 1) was cloned into a *Yarrowia* autonomously replicating vector to result in plasmid pY306 (SEQ ID NO:47), comprising a ColE1 plasmid origin of replication, an ampicillin-resistance gene, an f1 origin of replication and the *Y. lipolytica* Ura3 gene (GenBank Accession No. AJ306421).

[0216] The variant YlLPCAT sequence was synthesized by GenScript Corporation (Piscataway, NJ). Two internal *Nco*I restriction sites were removed by creation of silent mutations, while *Nco*I and *Not*I sites were added, respectively, at the 5' and 3' ends of the YlLPCAT open reading frame to facilitate cloning. Specifically, an A12T mutation (i.e., a change from adenosine [A] in YlLPCAT (SEQ ID NO:3) at position 12 to thymine [T] in the YlLPCAT variant) and a T918C mutation (i.e., a change from thymine [T] in YlLPCAT (SEQ ID NO:3) at position 918 to cytosine [C] in the YlLPCAT variant) were introduced into the YlLPCAT coding sequence. These two nucleotide substitutions were silent with respect to the amino acids encoded by the variant sequence. The nucleotide sequence encoding the variant YlLPCAT lacking its internal *Nco*I sites (i.e., YlLPCAT*) is represented by SEQ ID NO:45, while the amino acid sequence encoded thereby is represented by SEQ ID NO:46, which is identical to SEQ ID NO:4 (wild type YlLPCAT).

[0217] YlLPCAT* was subsequently cloned into plasmid pY306, thereby producing pY306-N (SEQ ID NO:48; FIG. 4). Thus, construct pY306-N contained the following components:

Table 9. Components of Plasmid pY306-N (SEQ ID NO:48)

RE Sites and Nucleotides within SEQ ID NO:48	Description of Fragment and Chimeric Gene Components
*BsiW*I/*BsiW*I	YAT1::YlLPCAT*::Lip1 (complementary), comprising:
1-2809	• YAT1: *Y. lipolytica* YAT1 promoter (U.S. Pat. Appl. Publ. No. 2010/0068789);
	• YlLPCAT*: variant *Y. lipolytica* acyl-CoA:lysophosphatidylcholine acyltransferase, lacking two internal *Nco*I sites (SEQ ID NO:45);
	• Lip1: Lip1 terminator sequence from *Yarrowia Lip1* gene (GenBank Accession No. Z50020)
*BsiW*I/*Eco*RI	• ColE1 plasmid origin of replication
2809-5605	• Ampicillin-resistance gene
	• f1 origin of replication
*Eco*RI/*Pac*I	*Y. lipolytica URA3* gene (GenBank Accession No. AJ306421)
5605-7021	

[0218] Plasmid pY306-N was used to prepare single- and double-mutants of YlLPCAT protein, as described below in Examples 4 and 6, respectively.

EXAMPLE 4

Designing and Synthesizing Mutant YILPCAT Enzymes with Modified Motifs

[0219] Based on the premise that conserved amino acid motifs within YILPCAT are likely involved in catalysis, it was concluded that generation of mutants having variant motifs could result in the identification of an LPCAT enzyme having improved functional activity.

[0220] A series of single amino acid substitutions were designed within the conserved sequence spanning amino acid residues 132 to 148 of SEQ ID NO:4 (i.e., Motif I) and the conserved sequence spanning amino acid residues 376 to 390 of SEQ ID NO:4 (i.e., Motif II). Within Motif I, a total of 195 amino acid substitutions were designed, as shown in Table 10, by creating various substitutions at each of the 17 amino acid residues within the motif.

Table 10. Single Amino Acid Substitutions within Motif I of YILPCAT Protein

Wild type residue	Single Amino Acid Substitutions	SEQ ID NO
M132	M132A, M132N, M132C, M132G, M132Q, M132H, M132I, M132L, M132F, M132P, M132S, M132T, M132W, M132Y and M132V	49
V133	V133A, V133N, V133C, V133G, V133Q, V133H, V133L, V133M, V133F, V133P, V133S, V133T, V133W and V133Y	50
L134	L134A, L134N, L134C, L134G, L134Q, L134H, L134M, L134F, L134P, L134S, L134T, L134W, L134Y and L134V	51
C135	C135R, C135N, C135D, C135G, C135E, C135Q, C135H, C135I, C135L, C135K, C135M, C135F, C135P, C135S, C135W and C135Y	52
M136	M136A, M136N, M136C, M136G, M136H, M136I, M136F, M136P, M136S, M136T, M136W, M136Y and M136V	53
K137	K137A, K137R, K137N, K137G, K137H, K137P, K137S, K137T, K137Y	54
L138	L138A, L138N, L138C, L138G, L138Q, L138H, L138I, L138M, L138F, L138P, L138S, L138T, L138W, L138Y	55
S139	S139A, S139N, S139C, S139G, S139H, S139L, S139M, S139F, S139P, S139W, and S139V	56
S140	S140N, S140C, S140H, S140I, S140L, S140F, S140P, S140W, S140Y and S140V	57
F141	F141A, F141N, F141G, F141H, F141I, F141M, F141P, F141S, F141T, F141W, and F141V	58
G142	G142N, G142H, G142I, G142L, G142M, G142F, G142P, G142T, G142W, G142Y and G142V	59
W143	W143A, W143G, W143H, W143L, W143K, W143P, W143S, W143T and W143V	60
N144	N144A, N144R, N144G, N144H, N144K, N144F, N144P, N144T and N144V	61
V145	V145A, V145C, V145G, V145E, V145H, V145M, V145F, V145P, V145S, V145T, V145W	62
Y146	Y146R, Y146N, Y146D, Y146G, Y146E, Y146Q, Y146I, Y146L, Y146M, Y146F, Y146P, Y146W and Y146V	63
D147	D147A, D147N, D147G, D147E, D147Q, D147H, D147F, D147S, D147T	64
G148	G148A, G148N, G148H, G148L, G148M, G148F, G148S, G148T and G148V	65

[0221] Similarly, a total of 134 amino acid substitutions were designed within Motif II, as shown in Table 11, by creating various substitutions within 12 of the 15 amino acid residues within the motif. No substitutions were made at W379, H380 and G381, since the histidine of other LPCATs corresponding to H380 of YILPCAT has been reported to be a likely active site residue (Lee et al., 2008, Mol. Biol. Cell 19:1174-1184).

Table 11. Single Amino Acid Substitutions within Motif II of YILPCAT Protein

Wild type residue	Single Amino Acid Substitutions	SEQ ID NO
S376	S376A, S376G, S376H, S376L, S376F, S376P, S376T and S376V	66
A377	A377N, A377G, A377H, A377L, A377F, A377P, A377S, A377T and A377V	67
F378	F378A, F378N, F378C, F378G, F378H, F378L, F378P, F378S, F378T, F378W, F378Y	68
T382	T382A, T382N, T382G, T382Q, T382H, T382I, T382M, T382P, T382S,	69

Wild type residue	Single Amino Acid Substitutions	SEQ ID NO
	T382W, T382Y	
R383	R383A, R383N, R383D, R383G, R383E, R383Q, R383H, R383I, R383L, R383K, R383M, R383F, R383P, R383T, R383W and R383V	70
P384	P384A, P384R, P384G, P384H, P384I, P384L, P384K, P384M, P384F, P384S, P384T, P384W, P384Y and P384V	71
G385	G385A, G385N, G385C, G385G, G385H, G385I, G385L, G385K, G385M, G385F, G385S, G385T, G385W, G385Y and G385V	72
Y386	Y386A, Y386G, Y386H, Y386L, Y386F, Y386P, Y386S, Y386T and Y386V	73
Y387	Y387A, Y387G, Y387H, Y387L, Y387F, Y387P, Y387S, Y387T, Y387W and Y387V	74
L388	L388A, L388G, L388H, L388P, L388S, L388T, L388W, L388Y and L388V	75
T389	T389A, T389C, T389G, T389H, T389I, T389L, T389M, T389F, T389P, T389S, T389W, T389Y and T389V	76
F390	F390A, F390N, F390C, F390G, F390H, F390L, F390M, F390P, F390S, F390T and F390V	77

[0222] Each of the 329 YILPCAT mutants set forth above in Tables 10 and 11 were individually synthesized and cloned into *Nco*I/*Not*I-cut pY306-N vector by GenScript Corporation (Piscataway, NJ).

EXAMPLE 5

Identifying Single Amino Acid Substitutions in YILPCAT Having Improved LPCAT Activity

[0223] The present example describes the transformation of each of the 329 pY306-N vectors comprising a YILPCAT mutant polynucleotide sequence (Example 4) into *Y. lipolytica* strain Y8406U2, followed by analysis of the lipid profiles of the transformants.

[0224] Improved LPCAT activity was indirectly evaluated, based on the observations set forth in U.S. Pat. Appl. Publ. No. 2010-0317882-A1 and summarized in Example 1 (above). Specifically, improved LPCAT activity within *Y. lipolytica* strain Y8406U2 transformants comprising a mutated YILPCAT was concluded based on an increase in the concentration of EPA as a weight % of TFAs ["EPA % TFAs"] and/or an increase in the conversion efficiency of the delta-9 elongase, when either factor was compared to the EPA % TFAs or the conversion efficiency of the delta-9 elongase, respectively, in *Y. lipolytica* strain Y8406U2 expressing the parent wild type YILPCAT protein.

Transformation of *Y. lipolytica* Strain Y8406U2

[0225] Strain Y8406U2 was transformed to individually express one of each of the pY306-N vectors containing a mutant YILPCAT prepared in Example 4. Y8406U2 is a *Ura*$^-$ strain of Y8406. Details regarding the development of strains Y8406 and Y8406U2 are provided in U.S. Pat. Appl. Publ. No. 2010-0317882-A1. Following transformation, cells were placed onto MM plates and then three individual transformants of each transformation were streaked on fresh MM plates and kept in a 30 °C incubator for two days. Cells from streaked plates were cultivated in 24-well blocks with 3 mL MM, and incubated for 2 days at 30 °C with shaking at 250 rpm. The cells were then collected by centrifugation and resuspended in 3 mL High Glucose Media ["HGM"] (High Glucose Media comprises per liter: 80 g glucose, 2.58 g KH_2PO_4 and 5.36 g K_2HPO_4, pH 7.5 (do not need to adjust)). The cells were incubated another 5 days at 30 °C with shaking at 200 rpm. After 5 days growth in HGM, 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 gas chromatography (GC).

Analysis of Lipid Profiles within *Yarrowia* Transformed for Expression of Single Mutants of YILPCAT

[0226] Tables 12 (Batch 1), 13 (Batch 2), 14 (Batch 3), 15 (Batch 4) and 16 (Batch 5) below show the fatty acid profiles and delta-9 elongase conversion efficiencies of individual Y8406U2 transformants comprising a plasmid for expressing a particular single-mutated YILPCAT (single amino acid substitution in Motif I or Motif II). These measurements were also made for certain controls: transformants comprising an empty vector ["EV"] (i.e., a replicating plasmid with no LPCAT gene [Batch #1 only]) or pY306-N (wild

type YILPCAT protein expression ["WT"]).

[0227] More specifically, each table summarizes the number of replicates analyzed for each particular transformant ["#"], the average concentration of each fatty acid as a weight percent of TFAs ["% TFAs"], the standard deviation for EPA % TFAs ["EPA SD"], and the delta-9 elongase conversion efficiency ["% Conv"]. The % Conv. was calculated for each transformant according to the following formula.: (EDA + HGLA + ARA + ERA + ETA + EPA) / (C18:2 + C18:3 + EDA + HGLA + ARA + ERA + ETA + EPA) * 100.

[0228] The measured fatty acids were 16:0 (palmitate), 16:1 (palmitoleic acid), 18:0 (stearic acid), 18:1 (oleic acid), 18:2 (linoleic acid), ALA (alpha-linolenic acid), EDA (eicosadienoic acid), DGLA (dihomo-gamma-linolenic acid), ARA (arachidonic acid), ETrA (eicosatrienoic acid), ETA (eicosatetraenoic acid) and EPA (eicosapentaenoic acid).

[0229] Comparison of each mutant's performance relative to the wild type YILPCAT control should only be made within the particular batch in which each mutant was analyzed (i.e., comparisons should not be made between Batch #1 and Batch #2, for example). Mutants shown in bold-face font and followed by a "+" were selected for further studies, as discussed below.

Table 12. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #1 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
EV control	6	2.8	0.5	2.6	4.6	19.2	1.8	2.8	2.6	0.6	1.4	2.6	**48.7**	0.2	**74**
WT	15	2.8	0.5	2.7	4.5	17.9	1.8	2.7	2.7	0.6	1.4	2.4	**50.4**	1.1	**75**
M132A	3	2.8	0.4	2.9	4.8	19.7	2.2	2.5	2.3	0.6	1.4	2.0	**49.3**	0.4	**73**
M132I	3	2.7	0.5	2.8	4.8	19.4	2.0	2.7	2.5	0.6	1.5	2.3	**48.6**	0.3	**73**
V133M	3	2.6	0.5	2.9	5.4	19.3	2.1	2.8	2.4	0.6	1.5	2.2	**49.0**	0.7	**73**
C135I	3	3.0	0.5	2.8	4.6	17.5	1.7	2.6	2.6	0.7	1.5	2.2	**50.7**	2.5	**76**
C135M	3	2.5	0.5	2.9	5.6	20.1	2.5	3.0	2.3	0.6	1.5	2.0	**47.8**	1.7	**72**
M136A	3	2.7	0.4	2.9	4.8	19.4	2.2	2.5	1.6	0.6	1.4	2.1	**49.6**	0.1	**73**
L138A	3	2.9	0.5	2.9	3.1	18.0	1.8	2.6	2.6	0.7	1.4	2.1	**50.5**	1.9	**75**
L138C	3	3.0	0.5	2.8	4.8	19.8	2.1	2.6	2.3	0.7	1.4	2.0	**48.6**	0.9	**72**
L138M	3	2.7	0.6	2.9	5.2	16.8	1.5	2.8	3.0	0.7	1.5	2.4	**51.0**	3.0	**77**
S139A	3	2.7	0.4	2.8	4.8	19.5	2.3	2.6	2.2	0.6	1.4	2.0	**48.8**	1.2	**73**
S139C	3	3.2	0.5	2.8	4.6	19.6	2.0	2.5	2.3	0.6	1.4	2.0	**48.8**	0.6	**73**
S139L	3	2.7	0.5	2.8	5.0	17.9	1.8	2.7	2.6	0.7	1.5	2.2	**50.7**	2.2	**75**
S139M	3	2.5	0.4	3.0	5.4	19.7	2.3	2.8	2.4	0.6	1.5	2.1	**48.6**	0.2	**72**
S140I	3	3.1	0.5	2.8	4.6	17.7	1.7	2.7	2.7	0.7	1.5	2.3	**50.1**	2.7	**76**
F141M +	3	2.8	0.7	2.7	4.9	14.8	0.9	2.8	3.4	0.8	1.6	2.6	**53.1**	0.5	**80**
G142I	3	3.1	0.6	2.7	5.0	18.3	1.8	2.9	2.6	0.7	1.5	2.3	**49.0**	3.1	**75**
G142L	3	2.5	0.5	2.8	5.5	19.2	2.0	3.0	2.5	0.6	1.6	2.3	**48.7**	1.1	**73**
W143L	3	2.7	0.5	2.8	5.1	17.9	1.8	2.8	1.6	0.6	1.5	2.3	**50.4**	2.0	**75**
N144H	3	2.7	0.6	2.6	4.7	18.9	1.8	2.8	2.7	0.6	1.6	2.8	**48.1**	1.6	**74**
N144K	3	2.7	0.5	2.8	5.3	17.7	1.8	2.8	2.7	0.6	1.5	2.2	**50.5**	3.2	**76**
V145C	3	3.0	0.4	2.8	4.7	19.6	2.1	2.5	2.3	0.6	1.4	2.0	**49.4**	0.5	**73**
V145M +	3	2.9	0.7	2.7	5.0	16.2	1.3	2.8	3.1	0.7	1.5	2.4	**51.4**	2.1	**78**
Y146D	3	3.0	0.5	2.8	3.3	19.6	2.0	2.5	2.4	0.7	1.4	2.1	**49.0**	0.6	**73**
Y146E	3	3.2	0.5	2.9	4.9	19.7	2.0	2.5	2.5	0.7	1.3	2.1	**48.8**	0.3	**73**
Y146I	3	3.0	0.5	2.8	5.4	20.0	2.3	2.8	2.3	0.6	1.5	2.1	**47.6**	2.3	**72**
Y146L	3	2.6	0.5	2.7	5.0	17.7	1.6	2.7	2.8	0.6	1.5	2.4	**50.8**	2.2	**76**
Y146M	3	2.6	0.5	2.7	5.2	18.1	1.9	2.7	2.7	0.7	1.5	2.1	**50.7**	1.8	**75**
D147E	3	3.2	0.5	2.8	4.7	18.3	1.7	2.7	2.7	0.7	1.5	2.2	**49.5**	0.2	**75**
F378A	3	2.6	0.4	2.9	4.8	19.5	2.3	2.5	2.2	0.6	1.4	2.0	**49.9**	0.3	**73**
T382A	3	2.7	0.5	2.8	5.1	19.8	2.2	2.8	2.4	0.6	1.4	2.2	**48.3**	1.7	**72**
R383A	3	2.9	0.6	2.8	3.6	17.8	1.5	2.9	2.8	0.7	1.4	2.3	**50.2**	1.5	**76**

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
R383D	3	3.3	0.5	2.9	5.0	19.6	2.0	2.5	2.4	0.7	1.4	2.1	**48.7**	0.8	**73**
R383I	3	3.1	0.5	2.8	4.6	18.6	1.7	2.6	2.6	0.7	1.5	2.3	**49.2**	0.5	**74**
R383K	3	2.5	0.5	2.7	5.4	20.1	2.4	3.1	2.3	0.6	1.5	2.1	**47.7**	2.6	**72**
R383L	3	2.5	0.4	2.8	5.0	19.6	2.1	2.7	2.4	0.6	1.5	2.1	**49.4**	0.4	**73**
R383M +	3	3.0	0.6	2.8	5.0	16.5	1.5	2.7	3.0	0.7	1.5	2.2	**52.2**	2.8	**78**
R383N	3	3.0	0.5	2.8	4.8	19.3	2.0	2.5	2.4	0.6	1.4	2.1	**49.2**	0.5	**73**
P384I	3	2.8	0.5	2.9	4.8	19.3	2.1	2.6	2.3	0.6	1.4	2.1	**49.3**	0.4	**73**
P384L	3	2.5	0.5	2.8	5.2	18.8	1.9	2.8	2.6	0.6	1.5	2.3	**49.6**	0.6	**74**
G385I	3	2.4	0.4	2.9	5.2	19.4	2.1	2.7	2.4	0.6	1.5	2.1	**49.2**	0.3	**73**
G385L	3	2.5	0.5	3.0	5.5	19.7	2.3	2.9	2.3	0.6	1.5	2.1	**48.4**	0.1	**72**
Y387A	3	2.7	0.4	2.9	4.5	19.6	2.1	2.5	2.4	0.7	1.3	2.0	**49.8**	0.2	**73**
L388A	3	2.6	0.5	2.8	4.8	19.9	2.1	2.5	2.5	0.7	1.3	2.3	**48.9**	1.4	**73**
T389I	3	2.5	0.5	2.8	5.1	19.7	2.1	2.7	2.4	0.6	1.5	2.2	**48.9**	0.8	**73**
T389L	3	2.5	0.4	2.9	5.2	19.9	2.3	2.7	2.3	0.6	1.5	2.0	**48.9**	0.3	**72**
F390L	3	2.5	0.4	2.9	5.3	19.7	2.3	2.7	2.3	0.6	1.5	2.1	**48.9**	0.4	**72**
Mutant AVG		2.8	0.5	2.8	4.9	18.9	2.0	2.7	2.5	0.6	1.5	2.2	49.5		74
Mutant SD		0.2	0.1	0.1	0.5	1.2	0.3	0.2	0.3	0.0	0.1	0.2	1.1		56

Table 13. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #2 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
WT	5	3.01	0.6	2.9	4.9	15.0	1.2	2.8	3.2	0.7	1.5	2.5	**52.9**	1.1	**79.7**
M132F	3	2.6	0.6	2.8	5.6	19.2	1.9	2.8	2.7	0.6	1.5	2.5	**48.7**	1.3	**73.6**
M132W	3	2.6	0.6	2.7	5.5	18.5	1.7	2.9	2.7	0.5	1.6	2.7	**48.6**	0.4	**74.4**
M132Y	3	2.6	0.6	2.7	2.3	18.9	1.8	2.8	2.7	0.5	1.6	2.8	**48.1**	1.0	**73.8**
V133F	3	2.6	0.5	3.0	5.6	19.5	2.3	2.8	2.5	0.5	1.5	2.3	**48.6**	0.4	**72.7**
V133W	3	2.5	0.5	2.8	4.2	19.7	2.1	2.9	2.5	0.5	1.5	2.4	**47.8**	1.1	**72.6**
L134F	3	3.0	0.6	3.1	5.8	16.7	1.4	3.3	3.0	0.6	1.6	2.6	**50.0**	2.2	**77.2**
L134V	3	3.1	0.6	2.8	5.0	15.4	1.1	2.8	3.1	0.7	1.6	2.5	**52.3**	0.3	**79.2**
L134W	3	2.6	0.7	2.5	5.1	16.2	0.9	3.0	3.4	0.8	1.5	2.7	**51.0**	1.9	**78.5**
L134Y	3	2.9	0.6	2.8	2.1	16.8	1.3	2.7	1.9	0.6	1.7	2.6	**50.8**	0.2	**76.9**
C135F	3	3.0	0.7	2.7	5.2	15.1	1.0	2.8	3.3	0.7	1.5	2.6	**52.5**	0.5	**79.7**
C135W	3	2.5	0.5	2.8	5.1	18.1	1.5	2.8	2.7	0.6	1.5	2.6	**49.9**	0.2	**75.4**
C135Y	3	2.5	0.6	2.9	5.4	18.1	1.5	3.0	2.7	0.6	1.6	2.8	**49.0**	0.4	**75.2**
M136F	3	2.8	0.6	2.8	5.1	16.6	1.2	2.8	3.1	0.7	1.6	2.5	**51.8**	0.3	**77.8**
M136S +	3	3.3	0.7	2.5	4.9	12.6	0.9	2.7	3.2	0.7	1.6	2.3	**55.0**	0.5	**82.9**
M136T	3	2.7	0.6	2.8	5.4	14.7	1.1	3.0	3.2	0.6	1.5	2.6	**52.7**	2.6	**80.1**
M136V +	3	3.6	0.7	2.7	5.2	13.0	0.9	2.7	3.3	0.7	1.5	2.5	**54.1**	0.7	**82.3**
M136W	3	2.8	0.6	2.7	4.9	15.3	1.1	2.8	3.2	0.6	1.6	2.6	**52.7**	0.2	**79.4**
L138F	3	2.4	0.6	2.9	5.3	16.4	1.3	3.0	3.0	0.6	1.6	2.8	**50.9**	2.0	**77.7**
L138W	3	2.8	0.6	2.8	5.1	16.2	1.2	2.8	3.1	0.6	1.5	2.5	**51.7**	0.4	**78.2**
L138Y	3	2.6	0.6	2.6	3.5	16.9	1.5	2.7	1.8	0.6	1.5	2.6	**51.2**	1.9	**76.7**
S139F	3	3.1	0.7	2.7	3.8	16.0	1.3	2.8	3.1	0.7	1.6	2.6	**50.9**	2.7	**78.1**
S139W	3	2.9	0.6	2.8	4.9	14.8	1.1	2.8	3.2	0.7	1.5	2.5	**53.2**	0.3	**80.1**
S140F	3	2.8	0.6	2.7	5.1	15.6	1.3	2.8	3.1	0.6	1.5	2.5	**52.2**	2.3	**78.7**
S140W	3	3.2	0.6	2.7	5.3	12.8	0.9	2.7	3.3	0.7	1.6	2.4	**54.6**	0.4	**82.7**

		% TFAs												EPA SD	% Conv.
Mutant +	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
S140Y	3	3.1	0.8	2.4	4.7	14.2	0.9	2.8	3.4	0.7	1.7	2.8	**52.5**	1.9	**80.9**
F141V	3	3.3	0.7	2.8	3.6	14.0	1.0	3.0	3.2	0.6	1.7	2.6	**52.8**	1.3	**81.0**
F141W +	3	3.1	0.7	2.8	5.1	14.1	1.0	2.8	3.3	0.7	1.6	2.5	**53.6**	0.3	**81.0**
G142F	3	2.7	0.7	2.5	3.5	16.7	1.2	2.9	3.1	0.7	1.6	2.7	**50.7**	1.4	**77.5**
G142V	3	3.1	0.7	2.7	5.0	15.0	1.1	2.8	3.3	0.7	1.6	2.6	**52.6**	0.2	**79.9**
G142W	3	2.9	0.7	2.5	4.7	15.3	1.0	3.0	3.3	0.7	1.7	2.9	**51.5**	1.1	**79.5**
G142Y	3	2.9	0.6	2.6	4.9	17.5	1.5	2.8	2.9	0.6	1.6	2.6	**50.1**	1.6	**76.1**
V145F	3	2.9	0.6	2.6	5.0	14.9	1.0	2.8	3.3	0.7	1.5	2.6	**52.9**	0.1	**80.0**
V145W +	3	3.0	1.0	3.0	5.0	15.0	1.0	3.0	3.0	1.0	2.0	3.0	**53.1**	0.1	**80.1**
F378S	3	2.8	0.6	2.6	4.9	16.2	1.2	2.8	3.0	0.6	1.5	2.5	**52.2**	0.2	**78.3**
F378T	3	2.7	0.7	2.6	4.9	15.8	1.2	3.0	3.0	0.6	1.6	2.8	**51.6**	0.1	**78.7**
F378Y +	3	3.0	0.7	2.6	3.5	14.4	1.0	2.7	3.4	0.7	1.6	2.7	**52.7**	1.0	**80.6**
T382P +	3	2.9	0.6	2.8	5.0	15.0	1.0	2.8	3.3	0.7	1.5	2.5	**53.0**	0.2	**79.9**
T382S	3	2.7	0.6	2.7	5.1	16.3	1.5	2.9	2.9	0.6	1.6	2.6	**51.3**	1.7	**77.6**
T382W	3	2.7	0.7	2.6	5.3	16.3	1.3	2.8	3.1	0.6	1.6	2.8	**51.1**	2.6	**77.9**
T382Y +	2	3.1	0.7	2.7	5.0	14.6	1.0	2.7	3.3	0.7	1.6	2.7	**52.8**		**80.3**
R383F	3	2.7	0.6	2.6	5.0	16.9	1.5	2.7	2.9	0.6	1.5	2.5	**51.4**	1.7	**77.1**
R383P	3	2.6	0.6	2.7	5.1	17.7	1.4	2.8	2.8	0.6	1.6	2.5	**50.4**	0.5	**76.1**
R383T	3	2.5	0.6	2.9	5.3	15.8	1.2	3.0	3.0	0.6	1.6	2.7	**51.9**	0.7	**78.7**
R383V	3	3.1	0.6	2.8	2.1	17.9	1.4	2.8	2.9	0.6	1.5	2.7	**49.2**	1.3	**75.5**
R383W	3	2.7	0.6	2.9	5.3	17.2	1.4	2.8	2.8	0.6	1.6	2.5	**50.8**	0.5	**76.7**
P384F	3	2.6	0.6	2.8	5.3	17.6	1.4	2.9	2.9	0.6	1.5	2.6	**50.0**	0.4	**76.2**
P384M	3	2.8	0.6	2.8	5.3	17.2	1.4	2.8	2.9	0.6	1.5	2.5	**51.1**	0.4	**76.8**
P384T	3	2.7	0.6	2.8	3.5	16.6	1.3	2.8	2.9	0.6	1.5	2.6	**51.6**	0.1	**77.6**
P384W	3	2.8	0.6	2.7	2.1	17.0	1.5	2.7	2.8	0.6	1.6	2.5	**50.9**	1.6	**76.8**
P384Y	3	2.8	0.7	2.6	3.7	17.6	1.4	2.9	3.0	0.6	1.7	2.8	**49.2**	0.7	**76.1**
G385F	3	2.5	0.5	3.0	5.5	18.5	1.8	2.8	2.6	0.6	1.5	2.5	**48.9**	0.1	**74.3**
G385M	3	2.7	0.5	3.2	5.8	19.2	2.1	2.9	2.5	0.6	1.6	2.3	**48.1**	0.2	**73.1**
G385W	3	2.9	0.6	2.8	5.1	18.9	2.0	2.8	2.4	0.5	1.7	2.4	**47.9**	0.4	**73.5**
G385Y	3	2.8	0.5	2.9	3.9	19.0	2.0	2.8	2.6	0.5	1.6	2.5	**48.4**	0.2	**73.6**
Y387V	3	2.9	0.5	2.9	5.1	17.8	1.5	2.7	2.7	0.6	1.6	2.4	**49.9**	0.2	**75.6**
Y387W	3	2.8	0.6	2.8	3.5	17.0	1.5	2.6	2.7	0.6	1.5	2.4	**51.3**	1.7	**76.8**
L388V	3	3.0	0.6	3.0	3.7	18.4	1.7	2.8	2.7	0.6	1.7	2.5	**48.8**	0.1	**74.5**
L388W	3	3.0	0.6	2.8	2.0	16.6	1.3	2.7	2.8	0.6	1.6	2.5	**51.2**	0.5	**77.5**
L388Y +	3	2.8	0.7	2.5	4.8	15.3	1.0	2.7	3.3	0.7	1.5	2.6	**52.9**	1.5	**79.7**
T389M	3	3.1	0.6	2.9	5.2	15.6	1.1	2.9	3.2	0.7	1.5	2.5	**52.0**	0.3	**78.9**
T389W	3	2.6	0.7	2.6	2.3	19.2	1.9	2.8	2.6	0.5	1.6	2.8	**47.3**	0.7	**73.2**
T389Y	3	2.7	0.5	2.8	3.9	18.7	1.8	2.9	2.6	0.5	1.6	2.6	**48.5**	0.2	**74.2**
Mutant AVG		2.8	0.6	2.7	4.6	16.5	1.3	2.8	2.9	0.6	1.6	2.6	51.0		77.5
Mutant SD		0.2	0.1	0.2	1.0	1.7	0.3	0.1	0.3	0.1	0.1	0.1	1.8		

Table 14. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #3 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
WT	3	2.9	0.6	2.7	4.6	14.4	1.0	2.6	3.0	0.6	1.5	2.5	**54.2**	0.5	**80.6**
M132C	3	2.8	0.6	2.6	4.6	18.0	1.5	2.6	2.8	0.5	1.6	2.7	**50.4**	0.2	**75.7**
M132L	3	2.9	0.6	2.8	5.0	18.7	1.8	2.6	2.5	0.5	1.6	2.4	**49.7**	0.5	**74.3**
M132Q	3	2.9	0.4	2.8	4.7	19.4	2.2	2.4	2.4	0.5	1.3	2.1	**50.1**	0.0	**73.1**
V133L	3	2.9	0.5	2.7	5.3	20.4	2.8	2.8	2.0	0.4	1.5	2.1	**48.1**	2.2	**71.1**
L134A +	3	3.1	0.7	2.5	4.6	14.2	1.0	2.6	3.2	0.6	1.5	2.5	**54.4**	0.7	**81.1**
L134M	3	3.2	0.6	2.7	4.6	15.9	1.5	2.4	2.8	0.6	1.4	2.3	**53.3**	2.9	**78.3**
C135L	3	3.3	0.6	3.0	4.9	15.9	1.5	2.4	2.7	0.6	1.5	2.2	**52.6**	4.4	**78.0**
M136I	3	3.1	0.6	2.7	4.7	16.2	1.7	2.5	2.6	0.5	1.5	2.2	**52.4**	3.2	**77.5**
M136Y	3	2.7	0.6	2.6	4.5	17.6	1.4	2.7	2.8	0.5	1.5	2.5	**51.1**	0.6	**76.3**
K137N +	3	3.4	0.7	2.6	4.7	13.2	1.0	2.7	3.2	0.6	1.5	2.4	**55.2**	0.8	**82.2**
K137R	3	3.0	0.6	2.6	4.6	17.1	1.3	2.7	2.8	0.6	1.6	2.6	**51.4**	0.3	**77.0**
L138Q	3	3.0	0.5	2.8	4.6	18.2	1.8	2.4	2.6	0.6	1.4	2.3	**51.0**	1.6	**75.0**
S139V	3	3.1	0.7	2.6	4.7	15.8	1.1	2.6	3.0	0.6	1.5	2.4	**53.1**	0.5	**78.9**
S140L	3	3.3	0.6	2.7	4.8	15.1	1.5	2.4	2.8	0.5	1.5	2.3	**53.8**	3.8	**79.2**
S140V	3	3.2	0.6	2.8	4.8	15.8	1.4	2.5	2.8	0.6	1.4	2.3	**53.2**	2.9	**78.4**
F141I	3	3.1	0.6	2.7	4.8	16.0	1.6	2.5	2.7	0.6	1.5	2.2	**53.0**	3.3	**78.0**
G142T	3	3.2	0.6	2.7	5.0	15.9	1.4	2.5	2.7	0.6	1.5	2.3	**52.7**	2.3	**78.3**
W143A	3	3.0	0.5	2.7	5.3	19.3	2.4	2.7	2.1	0.5	1.5	2.2	**48.8**	3.8	**72.7**
W143V	3	3.2	0.6	2.7	4.4	16.4	1.5	2.5	2.8	0.6	1.5	2.4	**52.5**	2.2	**77.6**
N144R	3	3.0	0.6	2.6	4.6	15.2	1.2	2.8	2.9	0.6	1.5	2.4	**53.5**	0.1	**79.5**
N144T +	3	3.3	0.7	2.6	4.7	13.6	0.9	2.6	3.2	0.6	1.5	2.4	**55.2**	0.1	**81.9**
V145E	3	3.1	0.7	2.6	4.6	14.3	1.0	2.5	3.2	0.6	1.5	2.5	**54.2**	0.7	**80.8**
Y146F	3	3.3	0.6	2.8	4.6	16.1	1.5	2.4	2.8	0.6	1.4	2.3	**52.9**	2.7	**78.1**
Y146Q	3	3.3	0.6	2.7	4.6	14.7	1.1	2.5	3.0	0.6	1.5	2.3	**54.1**	0.3	**80.3**
Y146R	3	3.2	0.5	2.7	4.6	16.4	1.6	2.4	2.6	0.5	1.5	2.2	**53.0**	3.2	**77.6**
Y146V	2	3.1	0.6	2.7	4.8	17.6	1.9	2.6	2.5	0.5	1.5	2.2	**50.7**		**75.5**
G 148A +	3	3.2	0.7	2.6	4.6	13.4	0.9	2.5	3.2	0.6	1.6	2.5	**54.9**	0.3	**82.0**
G148L	3	3.0	0.6	2.7	4.8	16.8	1.7	2.5	2.6	0.5	1.5	2.3	**52.2**	2.5	**77.0**
S376L	3	2.7	0.5	2.8	4.9	19.2	2.1	2.6	2.4	0.5	1.6	2.3	**49.2**	0.3	**73.4**
F378L	3	3.0	0.5	2.8	4.5	16.9	1.3	2.5	2.7	0.6	1.5	2.3	**52.3**	0.1	**77.2**
F378W	3	3.0	0.7	2.5	4.9	14.9	1.0	3.0	3.4	0.6	1.5	2.7	**53.0**	1.0	**80.2**
T382I +	3	3.3	0.7	2.6	4.7	12.9	0.9	2.4	3.2	0.6	1.4	2.4	**55.8**	0.5	**82.6**
T382M	3	2.9	0.5	2.7	4.5	16.9	1.7	2.6	2.6	0.5	1.5	2.3	**51.9**	2.8	**76.8**
R383E	3	3.1	0.4	2.9	4.7	19.7	2.4	2.3	2.2	0.5	1.3	2.1	**49.5**	0.5	**72.4**
R383H	3	2.9	0.6	2.6	4.8	16.5	1.2	2.7	2.9	0.6	1.6	2.5	**52.1**	0.4	**77.8**
R383Q	3	3.3	0.6	2.8	4.7	16.9	1.3	2.5	2.9	0.6	1.4	2.4	**51.5**	1.2	**77.1**
P384A +	3	3.2	0.7	2.6	4.4	15.0	1.1	2.6	2.9	0.6	1.6	2.4	**53.5**	0.7	**79.8**
P384S	3	3.3	0.6	2.7	4.6	15.9	1.2	2.7	2.9	0.6	1.5	2.4	**52.5**	0.9	**78.6**
P384T	3	2.9	0.5	2.8	5.1	19.4	2.3	2.5	2.2	0.5	1.5	2.3	**49.2**	0.4	**72.8**
P384V	3	2.8	0.6	2.7	4.8	17.4	1.5	2.6	2.7	0.5	1.5	2.4	**51.4**	0.2	**76.5**
G385A	3	2.8	0.5	2.9	5.0	19.2	2.2	2.7	2.3	0.5	1.6	2.3	**48.6**	0.8	**73.1**
G385C	3	3.0	0.5	2.9	5.2	19.9	2.4	2.5	2.2	0.5	1.6	2.2	**48.5**	0.8	**72.0**
G385V	3	3.0	0.5	2.9	5.3	19.7	2.3	2.6	2.2	0.5	1.5	2.2	**48.4**	0.7	**72.3**

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
Y387F	3	3.1	0.5	2.8	4.8	18.3	1.8	2.4	2.4	0.5	1.5	2.2	**50.8**	1.5	**74.8**
Y387L	3	3.2	0.6	2.7	4.4	17.3	1.4	2.6	2.6	0.5	1.6	2.3	**51.0**	1.2	**76.5**
T389A +	3	3.2	0.5	2.9	4.8	13.6	1.0	2.4	2.9	0.6	1.5	2.2	**55.4**	0.1	**81.6**
T389C +	3	3.2	0.6	2.7	4.4	13.6	1.0	2.5	3.1	0.6	1.5	2.4	**55.3**	0.3	**81.8**
T389S +	3	3.2	0.6	2.8	5.0	13.3	1.0	2.4	3.1	0.6	1.5	2.3	**55.2**	0.3	**82.0**
T389V	3	2.9	0.6	2.8	4.6	16.0	1.2	2.7	2.9	0.6	1.5	2.4	**52.8**	0.4	**78.6**
Mutant AVG		3.1	0.6	2.7	4.7	16.3	1.5	2.6	2.7	0.6	1.5	2.3	52.3	1.3	77.7
Mutant SD		0.2	0.1	0.1	0.2	1.9	0.4	0.1	0.3	0.0	0.1	0.1	2.0		3.0

Table 15. Lipid Composition and Delta-9 Elongase Conversion Efficiency in Batch #4 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA ETA**		**EPA**		
WT	6	3.0	0.6	2.7	4.5	14.4	1.0	2.5	3.1	0.6	1.5	2.3	**54.6**	0.8	**82.0**
M132G	3	2.6	0.6	2.7	5.5	19.6	1.9	2.6	2.4	0.4	1.5	2.3	**49.1**	1.8	**74.4**
M132H	3	2.6	0.5	2.9	5.1	19.4	2.4	2.5	2.3	0.4	1.5	2.2	**50.5**	0.1	**74.5**
M132N	3	2.4	0.5	2.6	4.9	18.6	1.8	2.6	2.7	0.5	1.5	2.7	**50.0**	1.6	**75.9**
V133A	3	2.8	0.5	2.8	4.6	17.0	1.3	2.5	2.8	0.6	1.5	2.2	**52.9**	0.5	**78.7**
V133C	3	2.6	0.6	2.7	4.4	15.5	1.1	2.5	3.0	0.5	1.6	2.3	**54.7**	0.1	**80.8**
V133G	3	2.9	0.7	2.9	5.6	17.8	1.5	3.3	2.8	0.5	1.6	2.3	**49.8**	3.2	**77.0**
V133H	3	2.6	0.5	2.9	4.8	18.4	1.8	2.5	2.4	0.4	1.5	2.2	**51.8**	0.1	**76.4**
V133N	3	2.6	0.6	2.7	4.6	18.0	1.4	2.4	2.8	0.5	1.4	2.4	**52.2**	2.0	**77.3**
V133Q	3	2.7	0.5	2.9	4.9	19.2	2.1	2.4	2.3	0.4	1.5	2.0	**51.0**	7.9	**75.0**
L134C	3	2.7	0.7	2.5	4.6	13.7	0.9	2.6	3.4	0.6	1.6	2.6	**55.0**	1.5	**83.2**
L134G +	3	3.0	0.7	2.7	4.4	14.1	1.0	2.5	3.0	0.5	1.7	2.1	**55.3**	0.6	**82.6**
L134H	3	2.5	0.6	2.6	4.5	16.7	1.3	2.5	2.8	0.5	1.6	2.6	**53.6**	0.3	**79.2**
L134N	3	2.8	0.5	2.7	4.6	16.6	1.4	2.4	2.7	0.5	1.5	2.2	**53.5**	2.8	**79.0**
L134Q	3	2.8	0.6	2.7	4.5	15.9	1.1	2.5	3.0	0.5	1.5	2.5	**54.3**	1.5	**80.4**
C135D	3	2.9	0.6	2.7	4.5	13.7	1.1	2.3	3.0	0.5	1.5	2.2	**56.5**	0.2	**83.1**
C135E	3	2.5	0.6	2.8	4.8	17.4	1.5	2.7	2.7	0.4	1.6	2.3	**52.2**	1.7	**78.0**
C135G	3	2.7	0.6	2.7	4.5	16.1	1.2	2.4	2.9	0.5	1.5	2.3	**54.0**	0.2	**80.0**
C135H	2	2.7	0.8	3.3	7.6	20.8	1.3	5.5	3.1	0.5	2.0	2.7	**42.1**	10.8	**72.7**
C135K	3	2.6	0.6	2.6	5.1	17.6	1.5	2.7	2.9	0.5	1.6	2.6	**51.8**	2.8	**77.7**
C135N	3	2.9	0.6	2.7	4.8	15.0	1.3	2.5	3.0	0.6	1.5	2.2	**54.3**	4.4	**81.0**
C135Q	3	2.8	0.6	2.8	4.5	16.2	1.2	2.5	2.8	0.5	1.6	2.3	**54.2**	0.5	**79.9**
C135R	3	2.5	0.5	2.7	5.1	19.2	2.0	2.6	2.6	0.5	1.5	2.3	**49.9**	0.2	**75.0**
M136C	3	3.0	0.7	2.6	4.8	14.6	1.0	2.9	3.3	0.6	1.5	2.3	**54.2**	1.3	**81.9**
M136G	2	3.1	0.6	2.7	4.5	12.5	0.9	2.4	3.1	0.6	1.5	2.3	**57.0**		**84.7**
M136H	3	2.8	0.6	2.7	4.7	17.3	1.5	2.6	2.6	0.5	1.6	2.3	**52.9**	0.7	**78.2**
M136N	3	3.0	0.5	2.8	4.6	15.6	1.5	2.4	2.8	0.5	1.4	2.1	**54.6**	4.1	**80.2**
K137A	3	2.9	0.5	2.9	4.4	15.8	1.4	2.4	2.8	0.6	1.4	2.2	**54.2**	3.5	**79.8**
K137G	3	2.9	0.6	2.7	4.5	14.3	1.0	2.5	3.1	0.5	1.4	2.2	**55.8**	0.5	**82.4**
K137H +	3	3.2	0.6	2.6	4.4	12.0	0.9	2.3	3.2	0.5	1.5	2.2	**58.6**	0.2	**85.6**
L138G	3	2.7	0.6	2.7	4.5	15.2	1.0	2.5	3.1	0.5	1.5	2.4	**54.8**	0.1	**81.3**
L138H	3	2.9	0.6	2.7	4.3	14.3	1.1	2.5	3.1	0.5	1.5	2.4	**55.8**	0.2	**82.4**

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA ETA**		**EPA**		
L138I	2	3.0	0.6	2.6	4.2	15.0	1.1	2.3	2.9	0.5	1.5	2.4	**56.1**		**81.7**
L138N	3	2.9	0.6	2.6	4.4	15.3	1.1	2.4	3.0	0.6	1.5	2.3	**54.6**	0.9	**81.1**
S139G	3	2.7	0.6	2.7	4.5	15.0	1.0	2.6	3.1	0.5	1.5	2.4	**54.8**	1.6	**81.4**
S139H	3	2.8	0.6	2.6	4.7	15.5	1.4	2.5	2.9	0.5	1.5	2.4	**54.4**	3.9	**80.5**
S139N	3	2.9	0.6	2.7	4.4	15.4	1.1	2.4	3.0	0.6	1.5	2.3	**54.7**	0.1	**81.0**
S140C	3	2.9	0.6	2.8	4.9	14.9	1.3	2.6	3.0	0.5	1.5	2.1	**54.4**	4.3	**81.1**
S140H +	3	3.1	0.6	2.6	4.3	12.1	0.9	2.4	3.2	0.5	1.5	2.3	**58.6**	0.5	**85.5**
S140N	3	3.0	0.6	2.7	4.3	13.5	0.9	2.3	3.1	0.6	1.5	2.2	**56.6**	0.1	**83.5**
F141A	3	3.0	0.6	2.8	4.2	14.3	1.0	2.4	3.1	0.6	1.4	2.2	**55.9**	0.2	**82.5**
F141G	3	2.7	0.5	2.6	4.7	16.9	1.3	2.6	2.8	0.5	1.5	2.2	**53.3**	0.9	**78.8**
F141H	3	2.4	0.5	2.6	4.8	18.0	1.7	2.6	2.6	0.4	1.5	2.5	**52.3**	2.2	**77.2**
F141N	3	2.8	0.6	2.6	4.8	16.7	1.4	2.6	2.7	0.5	1.6	2.2	**53.2**	0.9	**78.9**
G142H	2	2.8	0.7	2.6	4.2	14.3	0.9	2.4	3.2	0.5	1.5	2.7	**55.9**		**82.7**
G142N	3	2.4	0.7	2.3	4.6	15.5	1.0	2.6	3.4	0.5	1.6	3.0	**53.0**	0.9	**80.9**
W143G	3	2.7	0.6	2.7	4.8	16.5	1.4	2.6	2.8	0.5	1.5	2.2	**53.3**	3.1	**79.1**
W143H	3	2.9	0.6	2.7	4.4	15.2	1.1	2.5	3.0	0.5	1.6	2.5	**55.1**	0.4	**81.3**
W143K	3	2.8	0.6	2.6	4.8	16.5	1.3	2.6	2.7	0.5	1.6	2.3	**54.0**	0.3	**79.4**
N144A +	3	3.2	0.6	2.7	4.4	12.5	0.9	2.3	3.2	0.6	1.4	2.2	**57.5**	0.1	**84.8**
N144G	3	2.9	0.7	2.5	4.5	14.7	1.1	2.5	3.2	0.5	1.4	2.6	**54.5**	2.5	**81.8**
V145A	3	2.8	0.7	2.5	4.4	13.1	0.8	2.3	3.4	0.6	1.5	2.6	**56.0**	0.3	**84.1**
V145G	2	2.9	0.6	2.6	4.5	14.1	1.0	2.5	3.1	0.5	1.6	2.4	**55.5**		**82.7**
V145H	3	3.1	0.6	2.7	4.6	15.5	1.2	2.5	2.9	0.5	1.6	2.4	**54.5**	1.2	**80.7**
Y146G	2	2.8	0.6	2.7	4.6	14.4	1.0	2.6	3.2	0.6	1.5	2.5	**54.9**		**82.2**
D147A	3	2.8	0.6	2.6	4.6	15.6	1.4	2.5	2.9	0.5	1.6	2.3	**53.9**	4.0	**80.2**
D147G	3	2.4	0.6	3.2	6.5	20.5	1.9	4.2	2.7	0.4	1.8	2.4	**45.2**	7.2	**72.9**
D147H +	3	3.4	0.6	2.6	4.2	13.3	1.0	2.4	3.0	0.5	1.5	2.2	**57.5**	0.9	**83.9**
D147N	3	2.9	0.6	2.7	4.4	14.5	1.0	2.5	3.1	0.6	1.6	2.3	**55.1**	3.2	**82.1**
D147Q +	3	3.2	0.6	2.7	4.3	14.0	1.0	2.5	3.0	0.5	1.6	2.3	**56.6**	0.2	**83.0**
G148H	3	3.2	0.6	2.7	4.6	15.4	1.5	2.5	2.8	0.5	1.6	2.4	**54.3**	4.3	**80.5**
G148N +	3	3.0	0.7	2.7	4.7	13.4	1.0	2.5	3.2	0.6	1.6	2.3	**55.8**	0.8	**83.5**
S376A	3	2.9	0.6	2.8	4.6	16.9	1.3	2.5	2.8	0.6	1.5	2.3	**52.8**	1.9	**78.8**
S376G	3	2.6	0.5	2.7	5.1	17.8	1.5	2.8	2.7	0.5	1.4	2.3	**51.7**	1.9	**77.4**
S376H	3	2.8	0.6	2.7	4.9	19.0	2.2	2.5	2.4	0.4	1.6	2.5	**50.3**	0.5	**75.1**
A377G	3	2.6	0.7	2.7	5.0	17.3	1.3	2.8	2.9	0.5	1.6	2.5	**51.4**	1.8	**78.1**
A377H	3	3.0	0.5	2.8	5.0	19.5	2.4	2.5	2.2	0.4	1.6	2.3	**49.9**	0.1	**74.2**
A377L	3	2.6	0.5	2.8	5.7	19.6	2.4	2.7	2.2	0.4	1.5	2.2	**49.7**	1.0	**74.1**
A377N	3	2.7	0.6	2.7	5.3	19.1	2.1	2.7	2.3	0.4	1.7	2.2	**49.1**	0.2	**74.7**
F378C	3	2.8	0.6	2.8	4.8	16.4	1.3	2.7	2.8	0.5	1.6	2.2	**53.0**	1.0	**79.4**
F378G	3	2.8	0.6	2.8	4.6	15.6	1.1	2.5	2.9	0.5	1.5	2.3	**54.2**	0.1	**80.5**
F378H	3	2.8	0.5	2.8	4.7	17.3	1.7	2.6	2.5	0.4	1.5	2.2	**53.0**	3.1	**78.0**
F378N	3	2.6	0.6	2.8	4.7	17.0	1.3	2.5	2.8	0.5	1.6	2.3	**52.9**	0.4	**78.7**
T382G	3	2.5	0.5	2.9	4.8	18.2	1.7	2.5	2.5	0.4	1.4	2.3	**51.9**	1.5	**76.6**
T382H	3	2.8	0.6	2.8	4.6	17.3	1.5	2.5	2.6	0.4	1.5	2.4	**53.4**	0.5	**78.3**

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA ETA**		**EPA**		
T382N	3	2.6	0.5	2.9	5.2	19.4	2.2	2.6	2.3	0.4	1.5	2.0	**50.2**	0.5	**74.4**
T382Q	2	2.9	0.7	3.1	5.7	16.8	1.0	3.9	3.2	0.5	1.8	2.7	**50.0**		**78.8**
R383G	3	2.3	0.7	3.4	7.6	21.1	1.3	5.7	3.3	0.5	2.1	3.1	**41.2**	7.4	**72.3**
P384G +	3	2.5	0.6	2.6	4.5	15.5	1.1	2.5	3.1	0.5	1.5	2.5	**54.2**	0.2	**80.8**
P384H	3	2.7	0.6	2.7	4.5	16.3	1.2	2.5	2.8	0.5	1.5	2.4	**54.0**	0.5	**79.8**
P384K	3	2.7	0.6	2.5	4.9	17.7	1.7	2.5	2.5	0.4	1.6	2.3	**52.6**	2.3	**77.4**
P384R	3	2.7	0.6	2.7	4.5	16.1	1.1	2.4	3.0	0.6	1.4	2.4	**54.1**	0.9	**80.1**
G385G	3	2.8	0.6	2.7	4.5	14.1	1.0	2.6	3.1	0.5	1.6	2.4	**55.2**	0.1	**82.5**
G385H	3	2.6	0.5	2.8	5.3	19.1	2.2	2.6	2.4	0.4	1.6	2.4	**49.8**	0.6	**74.8**
G385K	3	2.6	0.5	2.8	5.4	19.3	2.1	2.6	2.4	0.4	1.6	2.4	**50.1**	0.4	**74.7**
G385N	3	2.5	0.5	2.7	5.3	19.5	2.0	2.7	2.6	0.4	1.5	2.4	**49.7**	1.2	**74.6**
Y386A	3	2.7	0.5	2.9	4.9	19.2	2.0	2.5	2.5	0.5	1.5	2.2	**50.1**	0.3	**74.9**
Y386G	3	2.5	0.5	3.0	5.2	19.3	2.2	2.6	2.3	0.4	1.6	2.0	**50.0**	0.4	**74.6**
Y386H	3	2.8	0.5	2.9	5.2	19.3	2.2	2.5	2.3	0.4	1.6	2.4	**50.0**	0.5	**74.6**
Y386L	3	2.6	0.5	2.9	5.4	19.1	2.2	2.7	2.3	0.4	1.6	2.2	**50.1**	0.2	**74.8**
Y387G	3	2.5	0.6	2.6	5.1	17.9	1.5	2.8	2.8	0.5	1.6	2.5	**51.0**	2.1	**77.2**
Y387H	3	2.9	0.6	2.6	4.5	16.5	1.2	2.5	2.8	0.5	1.5	2.5	**53.7**	2.1	**79.5**
L388G +	3	2.8	0.6	2.7	4.4	14.6	1.0	2.6	3.1	0.5	1.6	2.5	**55.5**	0.8	**82.2**
L388H	3	2.9	0.6	2.7	4.5	15.9	1.2	2.5	2.8	0.5	1.5	2.4	**54.7**	0.9	**80.3**
T389G	3	2.5	0.5	2.9	5.2	17.9	1.9	2.8	2.6	0.4	1.6	2.3	**51.2**	0.7	**76.8**
T389H	3	2.7	0.5	2.7	5.0	18.7	1.9	2.6	2.4	0.4	1.6	2.4	**51.3**	0.6	**75.8**
F390A	3	2.5	0.5	3.1	6.0	14.8	1.3	2.2	2.6	0.5	1.5	2.0	**54.4**	4.1	**81.3**
F390C	3	2.9	0.6	2.9	5.2	13.8	0.9	2.5	3.0	0.5	1.6	2.1	**55.5**	0.4	**83.0**
F390G +	3	2.6	0.4	3.3	5.7	14.6	1.2	2.2	2.5	0.4	1.4	1.8	**55.9**	0.3	**81.8**
F390H	3	2.7	0.5	2.7	4.7	18.3	1.8	2.5	2.4	0.4	1.5	2.2	**52.3**	0.7	**76.6**
F390N	2	2.8	0.6	2.6	4.4	15.2	1.0	2.4	3.1	0.6	1.5	2.3	**55.1**	0.2	**81.4**
Mutant AVG		2.8	0.6	2.7	4.8	16.4	1.4	2.6	2.8	0.5	1.5	2.3	53.1	1.5	79.3
Mutant SD		0.2	0.1	0.2	0.6	2.1	0.4	0.5	0.3	0.1	0.1	0.2	2.9		3.2

Table 16. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #5 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
WT	6	2.9	0.6	2.4	4.0	13.6	1.0	2.0	2.9	0.5	1.6	2.3	**58.3**	1.5	**82.2**
M132P	3	2.7	0.5	2.3	4.8	19.5	2.7	2.2	2.0	0.4	1.5	1.9	**52.1**	1.1	**73.0**
M132S	3	2.7	0.5	2.7	5.2	19.3	2.4	2.5	2.1	0.2	1.6	2.2	**51.0**	0.1	**73.3**
M132T	3	2.6	0.7	2.4	5.5	19.6	2.4	2.7	2.3	0.4	1.6	2.4	**50.1**	1.4	**73.0**
V133P	3	2.7	0.5	2.5	5.0	19.4	2.2	2.3	2.2	0.5	1.5	1.9	**51.3**	0.4	**73.4**
V133S	3	2.8	0.6	2.7	5.0	17.7	1.7	1.7	2.6	0.3	1.6	2.4	**52.4**	0.1	**75.9**
V133T	3	2.9	0.6	2.5	5.0	18.7	2.3	2.5	2.2	0.4	1.5	2.1	**52.0**	2.6	**74.3**
V133Y	3	2.5	0.5	2.5	4.8	19.0	2.3	2.2	2.2	0.4	1.4	2.2	**52.5**	0.2	**74.0**
L134P	3	2.5	0.5	2.3	4.4	18.9	2.4	2.0	2.1	0.4	1.5	2.1	**53.2**	0.4	**74.2**
L134S	3	2.8	0.6	2.7	5.6	19.9	2.6	2.6	2.2	0.2	1.6	2.1	**49.6**	6.0	**72.1**
L134T	3	2.8	0.5	2.6	5.3	20.0	2.8	2.5	1.9	0.3	1.5	1.9	**50.6**	0.5	**72.0**
C135P	3	2.5	0.5	2.3	4.2	18.2	2.0	1.9	2.3	0.4	1.5	2.3	**54.1**	0.6	**75.5**
C135S	3	3.0	0.6	2.6	4.6	15.4	1.3	2.5	2.8	0.5	1.6	2.4	**55.0**	0.7	**79.5**

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
M136P	3	3.0	0.6	2.2	3.7	12.6	0.9	1.8	2.8	0.5	1.5	2.3	**60.2**	0.7	**83.6**
K137P	3	2.6	0.5	2.4	4.3	17.8	2.1	2.1	2.3	0.4	1.4	2.1	**54.5**	3.5	**76.0**
K137S	3	3.0	0.7	2.5	4.4	14.0	1.1	2.5	3.1	0.5	1.7	2.5	**56.6**	0.5	**81.6**
K137T	3	2.9	0.6	2.4	4.7	18.0	2.3	2.3	2.2	0.4	1.6	2.1	**53.1**	4.4	**75.3**
K137Y	3	2.7	0.7	2.0	4.0	12.0	0.9	1.8	3.0	0.5	1.4	2.4	**60.7**	2.8	**84.4**
L138P	3	2.5	0.4	2.2	4.5	19.1	2.6	1.9	1.9	0.4	1.4	2.0	**53.7**	0.9	**73.9**
L138S	3	3.0	0.6	2.5	4.4	14.7	1.2	2.5	2.9	0.5	1.7	2.3	**56.2**	0.9	**80.6**
L138T	3	3.1	0.7	2.4	4.4	14.4	1.1	2.3	2.8	0.5	1.7	2.3	**56.7**	0.6	**81.0**
S139P	3	2.6	0.5	2.5	4.3	17.3	2.0	2.0	2.3	0.4	1.4	2.1	**54.9**	3.2	**76.5**
S140P	3	3.0	0.6	2.4	3.9	13.0	1.0	1.9	2.9	0.5	1.5	2.3	**59.7**	0.7	**83.1**
F141P	3	2.5	0.6	2.0	4.6	18.8	2.4	2.1	1.9	0.3	1.5	2.1	**53.1**	2.1	**74.2**
F141S	3	2.8	0.7	2.1	4.4	15.1	1.7	2.2	2.5	0.4	1.7	2.2	**56.6**	5.4	**79.6**
F141T	3	3.1	0.7	2.4	4.4	13.9	1.1	2.3	3.0	0.3	1.6	2.4	**57.1**	0.1	**81.6**
G142M	3	3.0	0.6	2.4	4.6	16.0	1.6	2.3	2.6	0.5	1.5	2.2	**55.3**	3.2	**78.5**
G142P	3	2.8	0.5	2.5	4.4	15.7	1.6	2.4	2.6	0.4	1.4	2.2	**55.7**	3.6	**79.0**
W143P	3	2.5	0.5	2.1	4.1	17.5	1.6	2.0	2.3	0.4	1.5	2.2	**55.5**	0.3	**77.0**
W143S	3	3.0	0.7	2.5	4.5	15.4	1.3	2.5	2.8	0.4	1.6	2.3	**55.5**	0.2	**79.6**
W143T	3	2.8	0.6	2.5	5.3	19.4	2.6	2.6	2.1	0.3	1.6	2.2	**50.1**	0.8	**72.9**
N144F	3	3.1	0.7	2.3	4.3	12.2	0.9	2.1	3.0	0.5	1.6	2.3	**59.4**	0.6	**84.0**
N144P	3	2.7	0.5	2.4	4.2	16.3	1.3	2.3	2.7	0.5	1.5	2.3	**55.7**	0.3	**78.7**
N144V	3	2.8	0.6	2.0	3.8	11.6	0.9	1.7	2.7	0.5	1.5	2.2	**61.9**	1.0	**85.0**
V145P	3	2.7	0.5	2.3	4.3	17.6	1.5	2.1	2.4	0.4	1.4	2.2	**54.7**	1.0	**76.8**
V145S	3	3.0	0.7	2.2	4.5	15.4	1.7	2.3	2.6	0.5	1.6	2.3	**55.9**	4.0	**79.3**
V145T	3	3.2	0.7	2.6	4.5	14.1	1.2	2.6	3.0	0.5	1.6	2.4	**56.0**	0.6	**81.3**
Y146N	3	2.7	0.6	2.1	4.0	15.4	1.5	1.8	2.4	0.4	1.4	2.2	**57.8**	3.6	**79.6**
Y146P	3	2.6	0.7	2.3	4.9	16.4	1.5	2.5	2.9	0.5	1.6	2.6	**53.7**	4.5	**78.0**
D147F	3	3.2	0.6	2.4	4.5	15.0	1.6	2.1	2.6	0.5	1.6	2.1	**56.2**	4.3	**79.8**
D147S	3	2.9	0.6	2.2	4.6	16.1	1.8	2.4	2.6	0.5	1.6	2.2	**55.1**	3.3	**78.2**
D147T	3	2.7	0.5	2.2	5.0	20.0	2.9	2.2	1.8	0.3	1.5	1.9	**51.5**	0.4	**72.1**
G148F	3	2.9	0.6	2.4	4.6	15.3	1.6	2.3	2.6	0.4	1.7	2.3	**55.6**	4.4	**79.4**
G148M	3	2.9	0.6	2.4	4.5	16.0	1.6	2.2	2.6	0.4	1.6	2.2	**55.2**	1.8	**78.5**
G148S	3	2.8	0.5	2.5	5.2	19.9	2.8	2.4	1.9	0.3	1.5	1.9	**51.0**	0.6	**72.2**
G148T	3	2.6	0.5	2.2	4.8	19.6	2.7	2.0	1.8	0.3	1.4	1.9	**52.7**	0.2	**73.0**
G148V	3	2.7	0.5	2.2	3.9	14.7	1.5	1.7	2.4	0.4	1.5	2.1	**58.8**	3.9	**80.5**
S376F	3	2.6	0.5	2.4	4.9	18.8	2.3	2.3	2.3	0.4	1.6	2.2	**51.8**	0.4	**74.1**
S376P	3	2.6	0.5	2.5	5.1	19.2	2.5	2.4	2.1	0.4	1.6	2.0	**51.7**	1.5	**73.5**
S376V	3	2.5	0.5	2.3	4.1	17.6	1.9	2.0	2.3	0.4	1.4	2.1	**55.4**	1.8	**76.5**
A377F	3	2.6	0.5	2.6	5.0	19.2	2.4	2.4	2.2	0.4	1.6	2.2	**51.2**	0.9	**73.5**
A377P	3	2.9	0.6	2.6	4.9	17.2	1.6	2.5	2.4	0.4	1.7	2.1	**52.7**	0.8	**76.8**
A377S	3	2.8	0.6	2.4	4.3	16.2	1.4	2.3	2.6	0.4	1.6	2.3	**55.5**	1.4	**78.6**
A377T	3	2.7	0.5	2.3	4.6	18.9	2.4	2.2	2.0	0.3	1.6	2.1	**52.6**	1.8	**74.0**
A377V	3	2.4	0.4	2.4	4.4	19.0	2.5	1.9	1.9	0.4	1.3	1.9	**54.0**	0.9	**74.1**
F378P	3	2.6	0.5	2.7	5.2	18.8	2.2	2.6	2.3	0.4	1.6	2.2	**50.9**	0.3	**74.0**
G385S	3	2.5	0.5	2.5	5.0	18.7	2.2	2.4	2.3	0.4	1.6	2.4	**51.8**	0.8	**74.4**
G385T	3	2.6	0.6	2.4	4.8	18.8	2.4	1.7	2.1	0.2	1.6	2.3	**52.2**	1.9	**74.0**
Y386F	3	2.9	0.9	2.1	4.7	16.5	1.3	2.3	2.6	0.4	1.6	2.4	**54.0**	2.7	**78.1**
Y386P	3	2.3	0.6	2.4	5.0	17.9	1.8	2.6	2.7	0.4	1.7	2.9	**51.3**	1.0	**75.8**
Y386S	3	2.7	0.6	2.6	5.3	19.2	2.3	2.5	2.2	0.4	1.6	2.2	**51.0**	0.2	**73.5**

		% TFAs												EPA SD	% Conv.
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**		
Y386T	3	2.6	0.6	2.6	5.5	19.5	2.2	2.7	2.3	0.4	1.7	2.4	**49.7**	1.6	**73.1**
Y386V	3	2.4	0.4	2.5	4.5	18.9	2.4	2.1	2.0	0.3	1.4	2.0	**53.3**	1.3	**74.1**
Y387P	3	2.8	0.6	2.7	4.7	17.1	1.6	2.5	2.5	0.4	1.7	2.3	**53.4**	0.1	**77.0**
Y387S	3	2.6	0.7	2.5	4.9	17.1	1.6	2.6	2.6	0.4	1.6	2.4	**53.4**	1.9	**77.2**
Y387T	3	2.7	0.6	2.4	4.7	17.0	1.5	2.4	2.6	0.4	1.5	2.3	**54.0**	0.4	**77.3**
L388P	3	2.5	0.6	2.5	5.0	18.3	1.9	2.5	2.5	0.3	1.7	2.5	**51.7**	0.8	**75.2**
L388S	3	2.8	0.6	2.5	4.8	17.9	1.9	2.4	2.3	0.4	1.5	2.2	**53.0**	1.5	**75.7**
L388T +	3	2.5	0.6	2.2	3.8	14.8	1.1	1.9	2.7	0.4	1.4	2.4	**58.6**	0.4	**80.8**
T389F	3	3.0	0.6	2.7	4.5	15.9	1.3	2.5	2.7	0.4	1.6	2.4	**54.9**	0.1	**79.0**
T389P	3	2.8	0.6	2.7	5.1	17.9	2.1	2.6	2.4	0.1	1.6	2.2	**52.4**	1.6	**75.4**
F390M	3	2.5	0.7	2.2	4.6	16.1	1.5	2.3	2.8	0.4	1.6	2.7	**54.3**	2.1	**78.5**
F390P	3	2.7	0.5	2.5	5.1	19.8	2.8	1.6	1.9	0.2	1.5	2.0	**51.3**	0.6	**72.2**
F390S +	3	2.8	0.5	2.9	5.9	12.9	1.1	2.1	2.4	0.4	1.5	1.8	**58.0**	0.5	**82.6**
F390T +	3	2.6	0.5	2.5	4.4	14.1	1.1	1.8	2.4	0.4	1.4	2.1	**59.2**	0.3	**81.6**
F390V	3	2.4	0.5	2.2	4.2	17.2	1.6	2.0	2.3	0.4	1.5	2.3	**55.6**	1.5	**77.3**
Mutant AVG		2.7	0.6	2.4	4.6	17.0	1.8	2.2	2.4	0.4	1.5	2.2	54.3	1.5	77.0
Mutant SD		0.2	0.1	0.2	0.5	2.3	0.6	0.3	0.3	0.1	0.1	0.2	2.8		3.4

[0230] Based on the above data, it was clear that several of the YlLPCAT single-amino acid mutants functioned with approximately equal or improved activity when compared to the parent wild type YlLPCAT enzyme (SEQ ID NO:46). This conclusion was made based on measuring LPCAT activity as a function of EPA % TFAs and/or % Conv. In fact, all of the mutant YlLPCAT transformants had an EPA % TFAs of at least 75% of the EPA % TFAs measured in the control (transformants with wild type YILPCAT). Also, all of the mutant YlLPCAT transformants had a % Conv. that was at least 87.6% of the % Conv. measured in the control.

[0231] Fifty-six (56) YlLPCAT mutants (comprising one of the following mutations with respect to SEQ ID NO:46: L134A, L134C, L134G, C135D, C135I, M136G, M136P, M136S, M136V, K137N, K137G, K137H, K137Y, L138A, L138H, L138M, S139L, S139W, S140N, S140H, S140P, S140W, F141A, F141M, F141W, G142H, W143L, N144A, N144K, N144F, N144T, N144V, V145A, V145G, V145E, V145M, V145F, V145W, Y146G, Y146L, Y146M, D147N, D147Q, D147H, G148A, G148N, T382I, T382P, R383M, L388G, L388Y, T389A, T389C, T389S and F390C) were found to exhibit equivalent or improved EPA % TFAs and equivalent or improved % Conv. An additional 14 YlLPCAT mutants were determined to have equivalent or improved EPA % TFAs when compared to the control (but did not have an equivalent or improved % Conv.), including mutants V133C, M136N, L138G, L138I, L138N, S139G, S139N, W143H, G148V, L388H, L388T, F390G, F390N and F390T. An additional 12 YlLPCAT mutants were determined to have equivalent or improved % Conv. when compared to the control (but did not have an equivalent or improved EPA % TFAs), including mutants C135F, M136T, S140Y, S140I, F141V, G142I, G142V, D147E, F378Y, T382Y, R383A and F390S.

[0232] A total of 26 YlLPCAT mutants, each comprising a single mutation within either Motif I or Motif II and having equivalent or improved EPA % TFAs and/or equivalent or improved % Conv. were selected for further evaluation (below, Example 6): L134A (100.4%, 100.6%), L134G (101.3%, 100.7%), M136S (104.0%, 104.0%), M136V (102.2%, 103.3%), K137H (107.3%, 104.4%), K137N (101.8%, 102.0%), S140H (107.3%, 104.3%), S140W (103.2%, 103.8%), F141M (105.4%, 106.7%), F141W (101.2%, 101.6%), N144A (105.3%, 103.4%), N144T (101.8%, 101.6%), V145M (102.0%, 104.0%), V145W (100.4%, 100.5%), D147H (105.3%, 102.3%), D147Q (103.6%, 101.2%), G148A (101.3%, 101.8%), G148N (102.2%, 101.8%), T382I (102.9%, 102.5%), T382P (100.2%, 100.2%), R383M (103.6%, 104.0%), L388G (101.6%, 100.2%), L388Y (100.0%, 99.9%), T389A (102.2%, 101.2%), T389C (102.1%, 101.5%), T389S (101.9%, 101.7%), where the first and second percentages in each parenthetical set correspond to the percentage ratio of EPA % TFAs and % Conv., respectively, in the mutant YlLPCAT transformants relative to the EPA % TFAs and % Conv. in the wild type YlLPCAT control transformants. An additional 8 YlLPCAT mutants, each comprising a single mutation within either Motif I or Motif II, also were selected for further evaluation (below, Example 6): F378Y (99.6%, 101.1%), T382Y (99.8%, 100.8%), P384A (98.7%, 99.0%), P384G (99.2%, 98.6%), L388T (100.5%, 98.3%), F390G (102.4%, 99.8%), F390S (99.4%, 100.5%) and F390T (101.6%, 99.3%), where the parenthetical sets are as above.

EXAMPLE 6

Identifying Double Amino Acid Substitutions in YILPCAT Having Improved LPCAT Activity

[0233] The present example describes the synthesis of double YILPCAT mutants, wherein the double mutants comprise both a single mutation within Motif I and a single mutation within Motif II. These double mutants were transformed into *Y. lipolytica* strain Y8406U2, followed by analysis of the lipid profiles of the transformants. As in Example 5, improved LPCAT activity was indirectly evaluated based on EPA % TFAs and % Conv.

Generation of Double YILPCAT Mutants

[0234] Preferred single mutations within Motif I (L134A, L134G, M136S, M136V, K137H, K137N, S140H, S140W, F141M, F141W, N144A, N144T, V145W, V145M, D147H, D147Q, G148A and G148N) were combined with preferred single mutations within Motif II (F378Y, T382I, T382P, T382Y, R383M, P384A, P384G, L388G, L388T, L388Y, T389A, T389C, T389S, F390G, F390S, F390T) to generate various combinations of double-mutant YILPCAT sequences. Thus, for example, a YILPCAT mutant comprising an S140W mutation within Motif I and a T382I mutation within Motif II is referred to herein as a YILPCAT mutant S140W_T382I. These double mutants were individually synthesized and cloned into *Nco*I-*Not*I cut pY306-N vector by GenScript Corporation (Piscataway, NJ); SEQ ID NO:42 represents the mutant YILPCAT proteins encoded by the cloned sequences.

Transformation of *Y. lipolytica* Strain Y8406U2 and Analysis of Lipid Profiles within pY306-N Transformants

[0235] The plasmids were transformed into *Y. lipolytica* strain Y8406U2 and transformants were subsequently grown and subjected to lipid analysis, as described in Example 5. Tables 17 (Batch 6), 18 (Batch 7), 19 (Batch 8) and 20 (Batch 10) show the fatty acid profiles and delta-9 elongase conversion efficiencies of individual transformants of Y8406U2. These measurements were also made for control transformants comprising pY306-N (wild type YILPCAT protein expression ["WT"]). The Tables are formatted as described in Example 5.

[0236] Comparison of each mutant's performance relative to the wild type YILPCAT control should only be made within the particular batch in which each mutant was analyzed (i.e., comparisons should not be made between Batch #6 and Batch #7, for example). Mutants shown in bold-face font and followed by a "+" were selected for further studies including flask assays, as discussed below.

Table 17. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #6 Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions

		% TFAs												EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
WT	6	2.7	0.7	2.3	5.6	14.4	0.9	3.0	3.1	0.7	1.5	2.7	**52.9**	0.2	80.6
S140W_T382I	3	2.9	0.8	2.2	5.8	13.0	0.8	2.9	3.2	0.7	1.5	2.7	**53.7**	1.2	82.4
S140W_T382P +	3	2.9	0.8	2.2	5.7	12.6	0.8	2.9	3.3	0.7	1.5	2.8	**54.3**	0.6	83.0
S140W_T382Y	3	2.7	0.7	2.2	5.6	13.6	0.9	2.8	3.2	0.7	1.5	2.8	**53.8**	0.6	81.8
S140W_R383M	3	2.9	0.7	2.3	5.8	12.6	0.8	2.9	3.3	0.8	1.5	2.6	**54.8**	0.6	83.1
S140W_P384A	3	2.8	0.7	2.3	5.7	13.9	0.9	2.9	3.1	0.7	1.5	2.7	**53.1**	1.3	81.2
S140W_L388Y	3	2.5	0.9	2.1	6.5	12.7	0.8	3.0	3.2	0.6	1.6	3.2	**52.9**	1.9	82.7
S140W_T389A +	3	2.4	0.7	2.2	6.5	11.6	0.7	2.5	3.1	0.7	1.5	2.6	**55.8**	0.4	84.3
S140W_T389C	3	2.7	0.7	2.3	6.0	12.6	0.8	2.8	3.4	0.8	1.5	2.7	**54.1**	0.4	83.0
S140W_T389S	3	2.6	0.6	2.5	6.3	14.6	1.3	2.7	2.7	0.7	1.5	2.2	**53.3**	4.1	79.9
M136V_F378Y +	3	2.5	0.7	2.2	4.0	14.7	1.3	2.8	2.9	0.7	1.5	2.6	**52.8**	4.3	79.8
M136V_T382I	3	2.5	0.7	2.3	6.1	14.5	1.2	2.9	2.9	0.7	1.6	2.8	**52.1**	4.5	80.0
M136V_T382P	3	2.7	0.8	2.2	5.6	12.8	0.8	2.9	3.3	0.8	1.6	2.8	**54.3**	0.4	82.8
M136V_T382Y	3	2.6	0.8	2.2	5.5	13.1	0.8	2.8	3.3	0.7	1.5	3.0	**54.3**	0.3	82.5
M136V_R383M	3	2.6	0.8	2.1	5.9	13.8	1.0	2.8	3.2	0.7	1.6	3.1	**52.3**	2.3	81.2
M136V_P384A	3	2.8	0.8	2.2	5.7	13.3	0.8	3.1	3.3	0.7	1.4	2.8	**53.2**	1.1	82.0

		% TFAs												EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
WT	6	2.7	0.7	2.3	5.6	14.4	0.9	3.0	3.1	0.7	1.5	2.7	**52.9**	0.2	80.6
M136V_L388Y	3	2.7	0.8	2.3	5.5	14.0	0.9	3.0	3.3	0.7	1.6	2.9	**53.0**	1.5	81.3
M136V_T389A +	3	2.7	0.7	2.4	6.1	11.8	0.8	2.6	3.0	0.7	1.4	2.3	**56.2**	0.4	84.0
M136V_T389S +	3	2.7	0.7	2.4	6.1	11.7	0.8	2.6	3.0	0.7	1.4	2.3	**56.5**	0.8	84.2
K137N_F378Y	3	2.8	0.8	2.2	5.5	13.6	0.9	2.9	3.3	0.7	1.5	2.8	**53.4**	1.1	81.7
K137N_T382I	3	2.4	0.8	2.2	6.0	15.0	1.3	2.8	3.0	0.6	1.6	2.9	**51.6**	4.7	79.3
K137N_T382P	3	2.4	0.9	2.0	3.6	13.1	0.8	2.8	3.4	0.7	1.5	3.4	**53.5**	1.7	82.5
K137N_T382Y	3	2.3	0.7	2.2	2.2	15.6	1.3	2.7	2.9	0.6	1.5	2.8	**51.5**	2.6	78.6
K137N_L388Y	3	2.2	0.8	2.1	3.7	14.9	1.1	2.9	3.0	0.6	1.6	3.1	**51.4**	3.0	79.6
K137N_T389C +	3	2.6	0.8	2.1	5.4	12.5	0.8	2.7	3.5	0.8	1.5	2.8	**55.1**	0.9	83.4
K137N_T389S +	3	2.5	0.7	2.3	6.0	11.8	0.7	2.6	3.2	0.7	1.5	2.5	**56.0**	0.2	84.2
N144T_F378Y	3	2.8	0.8	2.3	5.5	12.8	0.8	2.9	3.3	0.8	1.5	2.6	**54.4**	0.3	82.8
N144T_T382I	3	2.4	0.8	2.1	4.1	13.7	1.0	2.9	3.0	0.7	1.7	3.2	**52.4**	4.3	81.3
N144T_T382Y	3	2.5	0.8	2.3	3.7	13.8	0.9	2.9	3.2	0.7	1.5	2.8	**53.7**	0.2	81.6
N144T_R383M	3	2.5	0.8	2.1	5.2	12.7	0.8	2.7	3.3	0.7	1.5	2.8	**54.2**	0.1	82.9
N144T_T389A	2	2.4	0.7	2.4	5.8	12.5	0.8	2.7	3.3	0.7	1.6	2.7	**54.5**		83.2
N144T_T389C	2	2.2	0.8	1.7	4.8	11.9	0.8	2.3	3.1	0.7	1.6	2.8	**56.1**		84.0
N144T_T389S	3	2.5	0.6	2.3	5.9	12.0	0.7	2.7	3.2	0.7	1.7	2.5	**54.7**	0.7	83.7
V145W_F378Y	3	2.5	0.8	2.2	5.6	13.5	0.9	2.9	3.3	0.7	1.5	2.9	**52.6**	1.4	81.7
V145W_T382P	3	2.5	0.8	2.2	2.2	14.4	0.9	3.2	3.2	0.7	1.6	2.8	**52.5**	1.0	80.6
V145W_L388Y	2	2.7	0.8	2.3	3.3	16.1	1.3	3.0	2.7	0.6	1.6	2.6	**49.6**		77.5
V145W_T389A	3	2.5	0.7	2.4	6.1	13.5	1.0	2.9	3.1	0.7	1.5	2.7	**53.4**	1.3	81.6
V145W_T389C	3	2.6	0.7	2.4	3.9	15.3	1.3	2.9	2.9	0.7	1.5	2.6	**51.7**	3.5	79.0
V145W_T389S	3	2.7	0.6	2.5	4.2	14.1	1.0	2.8	3.1	0.7	1.5	2.5	**53.2**	0.7	80.9
Mutant AVG		2.6	0.7	2.2	5.2	13.4	0.9	2.8	3.2	0.7	1.5	2.8	53.6	1.6	81.8
Mutant SD		0.2	0.1	0.1	1.1	1.1	0.2	0.2	0.2	0.0	0.1	0.2	1.5	1.4	1.7

Table 18. Lipid Composition and Delta-9 Elongase Conversion Efficiency in Batch #7 Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions

		% TFAs												EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
WT	12	3.2	0.7	2.6	4.2	14.2	0.9	2.3	3.0		1.6	2.7	**54.1**	0.7	**81.0**
M136S_F378Y	3	3.4	0.7	2.6	4.7	12.0	0.8	2.1	3.0	0.7	1.6	2.5	**56.4**	1.3	**84.0**
M136S_T382I	3	3.4	0.8	2.6	5.2	11.2	0.8	2.2	2.9	0.6	1.6	2.6	**56.3**	1.2	**85.0**
M136S_T382P	3	2.9	0.8	2.3	4.5	11.5	0.7	2.1	3.3	0.6	1.5	3.1	**56.2**	1.6	**85.0**
M136S_T382Y	3	3.3	0.7	2.5	4.3	12.1	0.8	2.1	3.2	0.6	1.6	2.8	**55.8**	0.5	**84.0**
M136S_R383M	3	3.4	0.7	2.6	4.8	11.9	0.8	2.2	3.1	0.6	1.6	2.5	**56.1**	0.2	**84.0**
M136S_P384A	3	3.5	0.7	2.6	4.6	12.2	0.8	2.2	3.1	0.7	1.6	2.6	**56.1**	0.8	**84.0**
M136S_L388Y	3	3.3	0.7	2.5	4.3	12.2	0.8	2.3	3.2	0.6	1.6	2.6	**56.1**	1.5	**84.0**
M136S_T389A +	3	3.2	0.6	2.6	4.6	11.0	0.8	2.0	2.7	0.6	1.6	2.1	**57.9**	0.6	**85.0**
M136S_T389C +	3	3.3	0.6	2.7	4.8	11.2	0.8	2.1	3.0	0.7	1.6	2.3	**57.3**	0.2	**85.0**
M136S_T389S +	3	2.8	0.6	2.7	5.3	11.2	0.7	2.0	2.9	0.6	1.6	2.2	**57.7**	0.8	**85.0**
F141M_F378Y	3	3.0	0.7	2.5	3.9	13.5	0.9	2.4	3.1	0.6	1.6	2.6	**55.3**	0.4	**82.0**
F141M_T382I	3	3.1	0.7	2.7	4.4	16.2	2.2	2.2	2.3	0.5	1.7	2.8	**51.0**	4.6	**77.0**

		% TFAs											EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
F141M_T382P	3	2.9	0.7	2.6	4.2	14.5	1.1	2.3	3.0	0.6	1.6	2.6	**54.0**	0.7	**81.0**
F141M_T382Y	3	3.0	0.7	2.5	4.1	14.1	0.9	2.3	3.0	0.7	1.6	2.7	**54.2**	0.3	**81.0**
F141M_R383M	3	3.1	0.7	2.5	3.9	13.4	0.9	2.3	3.1	0.7	1.5	2.6	**55.3**	0.1	**82.0**
F141M_P384A	3	3.1	0.7	2.5	3.8	14.3	0.9	2.3	3.2	0.6	1.6	2.8	**54.5**	1.0	**81.0**
F141M_L388Y	3	3.0	0.6	2.5	4.2	17.3	1.6	2.4	2.5	0.6	1.6	2.5	**50.8**	3.7	**76.0**
F141M_T389A	3	3.2	0.6	2.8	4.3	14.5	1.3	2.3	2.7	0.6	1.6	2.2	**54.1**	2.1	**80.0**
F141M_T389C	3	2.9	0.7	2.5	4.0	13.3	0.9	2.3	3.1	0.7	1.5	2.7	**55.3**	0.1	**82.0**
F141M_T389S	3	2.8	0.6	2.7	4.8	15.8	1.4	2.5	2.8	0.6	1.6	2.4	**52.1**	4.4	**78.0**
F141W_F378Y	3	3.2	0.7	2.6	4.7	12.8	0.9	2.3	3.1	0.6	1.6	2.5	**55.5**	1.2	**83.0**
F141W_T382I +	3	3.0	0.7	2.5	4.6	11.7	0.8	2.1	3.2	0.7	1.5	2.5	**57.1**	0.5	**84.0**
F141W_T382P	3	3.3	0.8	2.6	4.2	13.5	0.9	2.3	3.2	0.7	1.5	2.7	**54.8**	1.6	**82.0**
F141W_T382Y	3	2.9	0.7	2.5	4.1	12.7	0.8	2.3	3.3	0.6	1.5	2.7	**56.0**	0.5	**83.0**
F141W_R383M	3	3.5	0.7	2.5	4.0	12.3	0.9	2.3	3.1	0.6	1.6	2.5	**56.1**	0.2	**83.0**
F141W_P384A	3	3.5	0.7	2.6	4.0	13.9	1.0	2.4	3.0	0.6	1.6	2.6	**54.3**	0.4	**81.0**
F141W_L388Y	3	3.2	0.7	2.7	4.3	14.2	1.0	2.4	3.0	0.6	1.5	2.6	**53.9**	0.8	**81.0**
F141W_T389A	3	3.3	0.6	2.8	4.6	12.3	0.9	2.1	2.9	0.6	1.6	2.2	**56.3**	0.4	**83.0**
F141W_T389C	3	3.3	0.7	2.8	4.4	12.5	1.0	2.4	3.0	0.6	1.4	2.4	**55.7**	0.8	**83.0**
F141W_T389S	3	3.1	0.6	2.7	4.4	12.5	0.9	2.2	3.0	0.6	1.5	2.4	**56.0**	1.2	**83.0**
V145M_F378Y	3	3.3	0.7	2.6	4.3	13.7	1.0	2.4	3.0	0.6	1.6	2.6	**54.0**	0.4	**81.0**
V145M_T382I	3	3.4	0.8	2.5	4.1	13.0	0.9	2.3	3.2	0.7	1.5	2.7	**54.9**	1.6	**82.0**
V145M_T382P	3	3.1	0.7	2.7	4.2	14.7	1.0	2.4	3.0	0.7	1.5	2.6	**53.5**	1.0	**80.0**
V145M_T382Y	3	3.6	0.7	2.7	4.3	14.4	1.0	2.3	3.0	0.6	1.6	2.6	**53.6**	2.7	**81.0**
V145M_R383M	3	3.4	0.7	2.5	4.0	13.3	0.9	2.3	2.9	0.6	1.6	2.4	**54.9**	0.6	**82.0**
V145M_P384A	3	3.2	0.8	2.4	3.9	15.4	1.0	2.4	2.8	0.6	1.7	2.8	**51.4**	3.6	**79.0**
V145M_L388Y	3	3.3	0.7	2.7	4.3	15.4	1.1	2.4	2.7	0.6	1.5	2.5	**52.2**	0.6	**79.0**
V145M_T389A	3	3.6	0.6	2.8	4.5	13.6	1.0	2.3	2.7	0.6	1.6	2.3	**54.1**	0.0	**81.0**
V145M_T389C	3	3.0	0.7	2.6	4.1	13.3	0.9	2.4	3.1	0.6	1.5	2.5	**55.4**	0.2	**82.0**
V145M_T389S	3	4.1	1.0	2.2	3.9	14.5	1.3	2.1	2.4	0.6	1.7	2.1	**51.5**	5.3	**79.0**
G148A_F378Y	3	3.3	0.7	2.6	4.3	12.5	0.9	2.3	3.1	0.6	1.5	2.5	**55.9**	0.3	**83.0**
G148A_T382I	3	3.3	0.7	2.6	4.7	11.8	0.8	2.3	3.1	0.6	1.6	2.5	**56.4**	0.5	**84.0**
G148A_T382P	3	2.9	0.6	2.6	4.4	15.1	1.2	2.4	2.9	0.6	1.6	2.7	**53.0**	3.7	**79.0**
G148A_T382Y	3	2.9	0.7	2.5	3.9	12.9	0.8	2.0	3.0	0.7	1.5	2.6	**56.1**	1.2	**83.0**
G148A_R383M	3	3.4	0.7	2.6	4.2	12.5	0.8	2.3	3.1	0.6	1.6	2.6	**55.5**	0.9	**83.0**
G148A_P384A	3	2.9	0.8	2.4	4.3	13.7	0.8	2.3	3.2	0.6	1.7	3.1	**53.7**	0.5	**82.0**
G148A_L388Y	3	2.7	0.8	2.3	4.0	13.8	0.9	2.4	3.2	0.6	1.6	3.0	**54.2**	0.5	**82.0**
G148A_T389A	3	3.0	0.6	2.7	4.8	12.5	0.8	2.2	3.0	0.6	1.5	2.4	**56.1**	0.2	**83.0**
G148A_T389C	3	3.5	0.7	2.6	4.2	12.6	0.9	2.3	3.0	0.6	1.5	2.4	**55.8**	0.1	**83.0**
G148A_T389S	3	3.3	0.6	2.8	4.7	14.8	1.3	2.4	2.7	0.6	1.6	2.3	**52.9**	5.0	**80.0**
Mutant AVG		3.1	0.7	2.6	4.4	13.2	1.0	2.3	3.0	0.6	1.6	2.6	54.9	1.4	80.0
Mutant SD		0.3	0.1	0.1	0.3	1.3	0.2	0.1	0.2	0.0	0.1	0.2	1.6		2.0

Table 19. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #8 Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions

		% TFAs											EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
WT	3	2.6	0.7	2.6	4.3	14.4	1.0	2.6	3.2	0.6	1.7	2.8	**53.8**	0.8	**81.0**
M136V_T389C+	3	2.8	0.6	2.6	4.8	12.1	0.9	2.3	3.3	0.6	1.5	2.6	**56.6**	0.5	**84.0**
K137N_R383M	3	2.8	0.7	2.5	4.4	12.9	0.9	2.4	3.3	0.6	1.5	2.8	**55.8**	0.4	**83.0**

		% TFAs											EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
K137N_P384A	3	2.6	0.6	2.7	4.9	17.7	1.9	2.8	2.6	0.6	1.6	2.5	**49.8**	4.2	**75.0**
K137N_T389A +	3	2.6	0.5	2.7	4.9	12.4	0.9	2.2	3.1	0.7	1.6	2.3	**56.8**	0.6	**83.0**
N144T_T382P	3	2.7	0.6	2.6	4.3	14.1	1.0	2.6	3.3	0.7	1.6	2.7	**54.4**	0.6	**81.0**
N144T_P384A	3	2.6	0.6	2.5	4.2	14.4	1.0	2.5	3.2	0.7	1.6	2.7	**54.3**	0.6	**81.0**
N144T_L388Y	3	2.5	0.7	2.4	3.9	14.0	0.9	2.4	3.4	0.7	1.5	3.0	**54.7**	0.7	**82.0**
V145W_T382I	3	2.9	0.6	2.6	4.7	13.0	0.9	2.5	3.3	0.7	1.5	2.6	**55.5**	0.3	**83.0**
V145W_T382Y	3	2.6	0.6	2.6	4.4	16.5	1.6	2.5	2.8	0.6	1.5	2.6	**52.1**	3.3	**77.0**
V145W_R383M	3	2.8	0.6	2.6	4.7	16.1	1.5	2.6	2.8	0.6	1.6	2.4	**52.3**	3.9	**78.0**
V145W_P384A	3	2.6	0.6	2.6	4.2	15.6	1.1	2.7	3.1	0.7	1.6	2.7	**52.7**	0.3	**79.0**
Mutant AVG		2.7	0.6	2.6	4.5	14.4	1.1	2.5	3.1	0.7	1.6	2.6	54.1	1.3	79.0
Mutant SD		0.1	0.1	0.1	0.3	1.7	0.3	0.2	0.3	0.1	0.1	0.2	2.1		2.8

Table 20. Lipid Composition and Delta-9 Elongate Conversion Efficiency in Batch #10 Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions

		% TFAs											EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
WT		2.9	0.7	2.7	4.2	14.6	1.1	2.6	3.0	0.6	1.5	2.6	**53.1**	1.7	80.1
L134A_T382I +		3.0	0.7	2.6	4.6	12.5	0.9	2.2	3.1	0.6	1.5	2.5	**55.9**	0.6	83.0
L134A_P384G		2.7	0.6	2.8	4.2	15.9	1.2	2.4	2.8	0.6	1.5	2.4	**52.7**	0.2	78.5
L134A_L388G		2.8	0.6	2.7	4.4	14.6	1.1	2.4	2.9	0.6	1.5	2.5	**53.9**	0.3	80.3
L134A_L388T		2.7	0.6	2.8	4.5	17.3	1.7	2.4	2.5	0.5	1.6	2.3	**51.0**	2.7	76.0
L134A_F390G		2.7	0.4	3.4	5.4	14.7	1.2	2.1	2.4	0.5	1.5	2.0	**53.6**	0.3	79.6
L134A_F390S		2.7	0.5	3.2	5.6	15.6	1.7	2.2	2.3	0.5	1.5	1.9	**52.5**	4.4	77.9
L134A_F390T		2.7	0.5	3.0	4.7	14.4	1.1	2.3	2.8	0.5	1.5	2.4	**54.2**	0.5	80.5
L134G_T382I		2.6	0.6	2.8	4.7	18.2	2.0	2.5	2.5	0.5	1.5	2.4	**49.6**	3.1	74.5
L134G_P384G		2.6	0.6	2.7	4.2	16.3	1.3	2.4	2.7	0.6	1.5	2.5	**52.4**	0.7	78.0
L134G_L388G		2.7	0.6	2.8	4.1	15.0	1.1	2.5	2.9	0.6	1.6	2.6	**53.4**	0.2	79.8
L134G_L388T		2.7	0.7	2.6	4.1	15.5	1.2	2.5	2.8	0.6	1.6	2.6	**52.4**	0.5	78.9
L134G_F390G		2.7	0.4	3.2	5.3	15.1	1.3	2.1	2.4	0.5	1.5	2.1	**53.3**	0.0	79.1
L134G_F390S		2.8	0.5	3.1	5.4	15.7	1.7	2.4	2.3	0.5	1.6	2.2	**52.0**	3.6	77.8
L134G_F390T		2.6	0.5	2.8	4.5	14.7	1.1	2.4	2.8	0.6	1.6	2.6	**53.5**	1.0	80.0
K137N_P384G		2.9	0.6	2.7	4.1	14.4	1.0	2.4	3.0	0.6	1.5	2.6	**54.2**	0.3	80.7
K137N_L388G		3.1	0.7	2.6	4.4	13.5	1.0	2.6	3.2	0.6	1.5	2.6	**54.5**	1.0	81.7
K137N_L388T		3.1	0.6	2.7	4.2	13.9	1.0	2.3	3.0	0.6	1.5	2.5	**54.8**	0.4	81.3
K137N_F390G +		2.4	0.5	3.0	5.5	13.1	0.9	1.9	2.7	0.5	1.5	2.4	**55.2**	0.9	82.1
K137N_F390S		2.8	0.5	3.2	5.5	13.9	1.1	2.1	2.6	0.5	1.5	2.1	**54.5**	1.2	80.9
K137N_F390T		2.8	0.6	2.9	4.6	14.1	1.0	2.2	2.7	0.6	1.6	2.3	**54.2**	0.4	80.9
K137H_T382I		3.1	0.6	2.8	4.7	14.8	1.5	2.2	2.7	0.5	1.5	2.3	**53.7**	4.7	79.4
K137H_P384G		2.7	0.8	2.4	4.1	13.3	0.9	2.3	3.3	0.6	1.6	3.0	**54.7**	0.3	82.2
K137H_L388G +		3.2	0.7	2.5	4.3	12.5	0.9	2.2	3.1	0.6	1.5	2.5	**56.2**	0.6	83.1
K137H_L388T +		3.1	0.7	2.7	4.3	13.0	0.9	2.2	3.0	0.6	1.5	2.5	**55.6**	0.1	82.5
K137H_F390G		2.8	0.5	3.3	5.7	14.6	1.2	2.0	2.5	0.5	1.5	2.1	**53.6**	1.2	79.7
K137H_F390S		2.6	0.6	3.1	6.0	12.9	1.0	2.1	2.6	0.5	1.6	2.4	**54.5**	0.8	82.1
K137H_F390T		2.8	0.5	2.9	4.9	14.0	1.0	2.2	2.8	0.5	1.5	2.5	**54.4**	0.6	81.0
S140H_T382I +		3.3	0.7	2.7	4.9	11.9	0.9	2.4	3.0	0.6	1.6	2.6	**55.4**	1.9	83.6

		% TFAs												EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
S140H_P384G		3.0	0.7	2.7	3.8	14.1	1.0	2.2	3.0	0.6	1.6	2.7	**54.5**	0.7	81.1
S140H_L388G +		3.0	0.7	2.5	4.2	12.7	0.8	2.3	3.2	0.6	1.5	2.7	**55.7**	0.1	83.0
S140H_L388T		3.2	0.7	2.5	4.1	13.2	0.9	2.4	3.0	0.6	1.7	2.6	**54.7**	0.4	82.1
S140H_F390G		2.6	0.5	2.8	5.5	13.9	1.0	2.0	2.7	0.5	1.6	2.6	**54.1**	1.2	81.0
S140H_F390S		2.8	0.5	3.1	5.2	14.1	1.1	2.2	2.6	0.5	1.5	2.2	**54.1**	0.4	80.6
S140H_F390T		3.0	0.6	2.9	4.7	16.0	1.3	2.5	2.7	0.5	1.6	2.5	**51.8**	1.4	78.1
N144A_T382I		3.1	0.6	2.7	4.8	14.5	1.5	2.2	2.7	0.5	1.6	2.4	**53.8**	5.3	79.8
N144A P384G		3.0	0.7	2.7	4.0	14.2	1.0	2.4	3.1	0.6	1.6	2.6	**54.1**	0.2	80.9
N144A_L388G		3.4	0.8	2.7	4.2	13.2	1.0	2.2	3.1	0.6	1.6	2.5	**54.7**	0.2	82.1
N144A_L388T		3.2	0.7	2.8	4.2	13.6	1.0	2.3	3.0	0.6	1.6	2.5	**54.6**	0.4	81.5
N144A_F390G		2.8	0.5	3.4	5.9	13.5	1.1	1.9	2.4	0.5	1.5	1.9	**54.6**	0.4	81.2
N144A_F390S +		2.7	0.5	3.2	6.0	12.8	1.0	1.9	2.5	0.6	1.5	2.0	**55.6**	1.2	82.3
N144A_F390T		2.8	0.6	2.9	4.7	13.9	1.0	2.2	2.8	0.6	1.5	2.5	**54.5**	1.1	81.1
D147Q_T382I		3.2	0.7	2.6	4.4	12.7	0.9	2.2	3.1	0.6	1.6	2.5	**55.6**	0.4	82.7
D147Q_P384G		2.9	0.6	2.7	4.1	16.4	1.3	2.5	2.7	0.6	1.7	2.5	**52.0**	0.2	77.8
D147Q_L388G		3.1	0.7	2.6	4.0	15.0	1.1	2.5	2.9	0.6	1.7	2.5	**53.4**	0.4	79.8
D147Q_L388T		2.7	0.7	2.6	4.0	15.1	1.1	2.3	2.9	0.6	1.6	2.7	**53.1**	0.1	79.7
D147Q_F390G		2.8	0.5	3.1	5.2	16.1	1.5	2.3	2.4	0.5	1.7	2.2	**51.7**	1.6	77.7
D147Q_F390S		2.7	0.5	3.1	5.1	14.0	1.1	2.2	2.5	0.6	1.5	2.1	**54.7**	0.7	80.9
D147Q_F390T		2.8	0.5	2.9	4.5	15.5	1.2	2.4	2.7	0.6	1.6	2.4	**52.8**	0.5	79.0
D147H_T382I +		3.2	0.7	2.6	4.6	12.4	0.9	2.3	3.1	0.6	1.6	2.4	**55.8**	0.1	83.2
D147H_P384G		2.7	0.7	2.5	3.9	15.0	1.0	2.4	3.1	0.6	1.8	2.8	**52.9**	0.5	79.9
D147H_L388G		2.9	0.7	2.6	4.3	14.1	1.0	2.4	3.0	0.6	1.6	2.6	**54.3**	0.3	81.1
D147H_L388T		2.8	0.6	2.6	4.2	14.4	1.0	2.4	3.0	0.6	1.6	2.6	**54.0**	0.2	80.7
D147H_F390G		2.8	0.5	3.1	5.4	15.4	1.3	2.2	2.5	0.5	1.5	2.2	**52.4**	2.2	78.6
D147H_F390S		2.8	0.5	3.1	5.6	13.7	1.1	2.1	2.6	0.5	1.5	2.1	**54.5**	0.5	81.1
D147H_F390T		2.8	0.5	2.9	4.6	14.8	1.1	2.4	2.8	0.5	1.6	2.5	**53.5**	0.4	79.9
G148A_P384G		2.7	0.8	2.5	4.1	14.6	0.9	2.4	3.3	0.6	1.7	3.1	**53.1**	0.4	80.6
G148A_L388G		3.1	0.7	2.7	4.1	14.1	1.1	2.5	3.0	0.6	1.6	2.6	**54.3**	0.4	81.0
G148A_L388T +		3.2	0.7	2.9	4.7	16.7	1.9	2.8	2.4	0.5	1.7	2.5	**50.2**	3.4	76.3
G148A_F390G		2.9	0.5	3.2	5.3	16.4	1.8	2.2	2.2	0.4	1.5	2.0	**51.7**	4.4	76.8
G148A_F390S +		2.6	0.5	3.3	5.8	12.3	1.0	2.1	2.6	0.5	1.5	2.0	**56.1**	0.3	82.9
G148A_F390T		3.0	0.5	3.0	4.6	14.0	1.1	2.2	2.6	0.5	1.6	2.3	**54.7**	0.2	80.9
G148N_T382I +		3.6	0.7	2.7	4.3	10.6	0.7	2.2	3.2	0.6	1.4	2.5	**58.5**	3.2	85.8
G148N_P384G		2.7	0.6	2.7	4.0	15.0	1.1	2.5	2.9	0.6	1.5	2.6	**53.5**	0.3	79.8
G148N_L388G		2.9	0.7	2.6	4.5	15.0	1.1	2.7	3.2	0.6	1.6	2.9	**52.2**	3.3	79.7
G148N L388T		2.8	0.6	2.7	4.1	14.4	1.1	2.5	3.0	0.6	1.6	2.7	**54.0**	0.7	80.6
G148N_F390G		2.5	0.4	3.2	5.7	13.6	1.1	2.0	2.5	0.5	1.4	2.0	**55.3**	0.3	81.3
G148N_F390S +		2.5	0.4	3.2	6.0	12.4	1.0	2.0	2.6	0.5	1.4	2.0	**56.2**	0.2	82.8
G148N_F390T		2.7	0.5	3.0	4.8	16.2	1.7	2.4	2.6	0.5	1.5	2.5	**52.0**	3.8	77.4
Mutant AVG		2.9	0.6	2.8	4.7	14.3	1.1	2.3	2.8	0.6	1.6	2.4	53.9	1.1	80.4

[0237] Based on the data set forth above, it is clear that most of the 167 YILPCAT double mutants analyzed above functioned with approximately equal or improved activity when compared to the parent wild type enzyme (SEQ ID NO:46). This conclusion was made based on measuring LPCAT activity as a function of EPA % TFAs and/or % Conv.

[0238] More specifically, 106 YILPCAT mutants comprising a single amino acid mutation within Motif I and a single amino acid mutation within Motif II were found to exhibit equivalent or improved EPA % TFAs and equivalent or improved % Conv. These mutants were L134A_T382I, L134A_L388G, L134A_F390T M136S_F378Y, M136S-T382I, M136S_T382P, M136S_T382Y, M136S_R383M, M136S_P384A, M136S_L388Y, M136S_T389A, M136S_T389C, M136S_T389S, M136V_T382P, M136V_T382Y, M136V_P384A, M136V_L388Y, M136V_T389A, M136V_T389C, M136V-T389S, K137H_P384G, K137H_L388G, K137H_L388T, K137H_F390S, K137H_F390T, K137N_T382P, K137N_R383M, K137N_P384G, K137N_F378Y, K137N_L388G, K137N_L388T, K137N_T389A, K137N_T389C, K137N_T389S, K137N_F390G, K137N_F390S, K137N_F390T, S140H_T382I, S140H_P384G, S140H_L388G, S140H_L388T, S140H_F390G, S140H_F390S, S140W-T382I, S140W-T382P, S140W-T382Y, S140W_R383M, S140W_P384A, S140W_L388Y, S140W_T389A, S140W_T389C, F141M_F378Y, F141M_T382Y, F141M_R383M, F141M_P384A, F141M_T389C, F141W_F378Y, F141W_T382I, F141W_T382P, F141W_T382Y, F141W_R383M, F141W_P384A, F141W_T389A, F141W_T389C, F141W_T389S, N144A_P384G, N144A_L388G, N144A_L388T, N144A_F390G, N144A_F390S, N144A_F390T, N144T_F378Y, N144T_T382P, N144T_T382Y, N144T_R383M, N144T_P384A, N144T_L388Y, N144T_T389A, N144T_T389C, N144T_T389S, V145M_T382I, V145M_R383M, V145M_T389A, V145M_T389C, V145W_T382I, D147H_T382I, D147H_L388G, D147H_L388T, D147H_F390S, D147Q_T382I, D147Q_F390S, G148A_F378Y, G148A_T382I, G148A_T382Y, G148A_R383M, G148A_P384G, G148A_L388G, G148A_L388Y, G148A_T389A, G148A_T389C, G148A_F390S, G148A_F390T, G148N_T382I, G148N_L388T, G148N_F390G and G148N_F390S).

[0239] An additional 15 YILPCAT double mutants (of the 167 analyzed) had equivalent or improved EPA % TFAs when compared to the control, while an additional 6 YILPCAT double mutants (of the 167 analyzed) were determined to have equivalent or improved % Conv. when compared to the control.

Confirmation of Improved LPCAT Activity by Flask Assay

[0240] A total of 23 YILPCAT double mutants, each comprising a single amino acid mutation within Motif I and a single amino acid mutation within Motif II, and having equivalent or improved EPA % TFAs and/or equivalent or improved % Conv., were selected for further evaluation (these mutants are noted in bold and with a "+" in Tables 17-20). These mutants were: S140W_T382P, S140W_T389A, M136V_T389A, M136V_T389C, M136V_T389S, K137N_T389A, K137N_T389C, K137N_T389S, M136S_T389A, M136S_T389C, M136S_T389S, F141W_T382I, L134A_T382I, K137N_F390G, K137H_L388G, K137H_L388T, S140H_T382I, S140H_L388G, N144A_F390S, D147H_T382I, G148A_F390S, G148N_T382I and G148N_F390S. Additionally, mutants M136V_F378Y and G148A_L388T, each having slightly diminished EPA % TFAs and slightly diminished % Conv. in comparison to the control, were selected for further evaluation.

[0241] Transformants expressing these double mutant YILPCAT proteins were subjected to flask assays for a detailed analysis of the total lipid content and composition. Specifically, the double mutant strains were individually inoculated into 3 mL FM in 15-mL Falcon™ tubes and grown overnight at 30 °C and 250 rpm. The OD_{600nm} was measured and an aliquot of the cells was added to a final OD_{600nm} of 0.3 in 25 mL FM medium in a 125-mL flask. After 2 days in a Multitron shaking incubator at 250 rpm and at 30 °C, 6 mL of the culture was harvested by centrifugation and resuspended in 25 mL HGM in the original 125-mL flask. After 5 days in a shaking incubator at 250 rpm and at 30 °C, water was added to flasks to bring the total volume back to 25 mL (thereby accounting for evaporation). An aliquot was used for fatty acid analysis (above) and 10 mL of the culture was dried for dry cell weight determination.

[0242] 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.

[0243] The flask assay results are shown below in Tables 21 (Group I) and 22 (Group II). The Tables summarize the number of replicates analyzed for each particular transformant ["#"], the average total dry cell weight of the cells ["DCW"], the average total lipid content of the cells ["TFAs % DCW"], the average concentration of each fatty acid as a weight percent of TFAs ["% TFAs"], the delta-9 elongase conversion efficiency ["% Conv."] and the average EPA content as a percent of the dry cell weight ["EPA % DCW"].

Table 21. Total Lipid Content, Composition and Delta-9 Elongate Conversion Efficiency in Selected Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions, by Flask Assay (Group I)

Mutant	#	DCW (g/L)	TFAs% DCW	% TFAs												% Conv.	EPA % DCW
				16:0	16:1	18:0	18:1	LA	ALA	EDA	DGLA	ARA	ETrA	ETA	EPA		
WT	2	3.7	26.0	2.7	0.7	2.6	4.8	13.7	1.1	2.5	3.5	1.0	0.7		**53.9**	**81.3**	14.0
S140W_T382P	2	3.9	28.6	2.7	0.7	2.5	5.2	11.8	0.9	2.6	4.0	1.1	0.9	3.3	**54.2**	**83.8**	15.5
S140W_T389A	2	4.0	28.2	2.7	0.6	2.8	6.1	11.7	0.9	2.4	3.4	0.9	0.6	2.5	**55.5**	**83.7**	15.7
M136V_F378Y	2	4.0	27.7	2.9	0.7	2.5	5.4	12.0	0.9	2.7	3.7	1.0	0.7	3.0	**54.2**	**83.4**	15.0
M136V_T389A	2	4.1	27.1	2.8	0.6	2.8	5.9	12.0	1.0	2.5	3.3	1.0	0.7	2.6	**54.6**	**83.3**	14.8
M136V_T389C+	2	4.0	27.3	3.0	0.5	2.7	5.0	11.6	1.0	2.6	3.3	1.0	0.6	2.6	**56.2**	**84.0**	15.4
M136V_T389S	2	4.0	28.2	2.8	0.6	2.8	5.8	11.7	1.0	2.5	3.3	1.0	0.7	2.6	**54.8**	**83.7**	15.5
K137N_T389A	2	3.8	25.8	3.0	0.5	3.0	5.6	12.1	1.1	2.4	3.1	0.9	0.6	2.3	**55.8**	**83.2**	14.4
K137N_T389C	2	4.0	27.4	2.8	0.8	2.5	5.4	13.2	1.0	2.8	3.8	1.0	0.6	3.1	**53.2**	**81.9**	14.6
K137N_T389S	2	3.9	27.2	2.7	0.7	2.7	6.0	12.3	1.0	2.6	3.5	0.9	0.6	2.6	**54.8**	**83.0**	14.9
M136S_T389A+	2	3.9	27.7	2.7	0.6	2.8	5.9	11.7	1.0	2.5	3.3	0.9	0.6	2.5	**55.8**	**83.9**	15.5
M136S_T389C+	2	3.9	26.9	3.0	0.5	2.8	5.3	11.7	1.0	2.5	3.3	0.9	0.7	2.6	**56.0**	**83.9**	15.1
M136S_T389S+	2	3.7	27.7	2.8	0.6	2.9	5.8	11.4	1.0	2.3	3.1	1.0	0.7	2.4	**55.8**	**84.1**	15.5
F141W_T382I	2	3.8	28.7	2.5	0.8	2.5	5.7	11.9	0.8	2.6	4.2	1.0	0.7	3.4	**53.4**	**83.7**	15.3

Table 22. Total Lipid Content, Composition and Delta-9 Elongate Conversion Efficiency in Selected Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions, by Flask Assay (Group II)

Mutant	#	DCW (g/L)	TFAs % DCW	% TFAs												% Conv.	EPA % DCW
				16:0	16:1	18:0	18:1	LA	ALA	EDA	DGLA	ARA	ETrA	ETA	EPA		
WT		2.0	26.0	3.0	0.7	2.5	4.2	13.7	0.9	2.4	3.4	0.7	0.5	3.5	**54.7**	**82**	14.2
L134A_T382I		2.0	24.0	3.3	0.7	2.6	4.4	12.6	0.9	2.2	3.5	0.8	0.6	3.5	**53.3**	**83**	12.9
K137N_F390G		2.1	27.3	2.1	0.4	2.5	6.2	12.4	0.9	1.9	3.7	0.8	0.8	3.8	**54.1**	**83**	14.8
K137H_L388G		2.0	28.1	3.2	0.7	2.4	4.3	12.6	0.9	2.4	3.5	0.8	0.6	3.5	**54.6**	**83**	15.4
K137H_L388T		2.0	27.4	2.9	0.7	2.4	4.4	13.2	0.9	2.4	3.6	0.7	0.6	3.5	**54.8**	**82**	15.0
S140H_T382I		2.1	21.3	3.4	0.9	2.6	4.8	12.6	0.9	2.4	3.7	0.7	0.5	3.6	**52.7**	**82**	11.3
S140H_L388G		2.0	26.1	2.7	0.8	2.2	4.4	13.0	0.9	2.5	3.9	0.7	0.6	4.0	**54.3**	**83**	14.2
N144A_F390S+		2.1	26.2	2.6	0.4	2.8	6.7	12.0	0.8	1.9	3.2	0.7	0.5	3.1	**55.9**	**84**	14.7
D147H_T382I		2.1	26.6	3.0	0.7	2.3	4.6	12.4	0.9	2.4	3.6	0.8	0.5	3.7	**54.3**	**83**	14.4
G148A_F390S+		2.1	27.0	2.8	0.4	3.0	6.5	12.0	0.8	2.1	2.9	0.8	0.7	3.0	**55.1**	**83**	14.9
G148N_T382I+		1.9	26.5	3.3	0.7	2.3	4.7	12.2	0.8	2.3	3.5	0.8	0.6	3.5	**56.7**	**84**	15.0
G148N_F390S+		2.1	26.7	2.8	0.4	2.9	6.5	12.0	0.8	2.0	3.0	0.7	0.6	2.9	**55.9**	**84**	14.9
G148A_L388T		2.0	24.7	2.5	0.6	2.2	5.4	11.7	0.9	2.2	3.6	0.8	0.5	3.7	**55.1**	**84**	13.6

[0244] Of the 25 YILPCAT double mutants analyzed, each comprising a single amino acid mutation within Motif I and a single amino acid mutation within Motif II, 17 were observed to have both equivalent or improved EPA % TFAs and equivalent or improved % Conv., while the remaining 8 had equivalent or improved % Conv.

[0245] Based on the data set forth above, 22 of the 25 YILPCAT double mutants analyzed above functioned with improved activity when compared to the parent wild type enzyme (SEQ ID NO:46).

[0246] Also, the over-expression of certain double-mutant LPCAT polypeptides resulted in increased total lipid content (TFAs % DCW) in the recombinant *Yarrowia.* For example, over-expression of mutant LPCAT polypeptides comprising the S140W_T382P, S140W_T389A, M136V_T389S and F141W_T382I, or K137H_L388G mutation pairs resulted in total lipid contents that were 8% or more increased relative to the total lipid content of the control (Tables 21 and 22). Interestingly, certain transformants had both

increased total lipid content and EPA % TFAs. For example, transformants that over-expressed LPCATs with S140W_T389A, M136V_T389C, M136S_T389A, or M136S_T389S mutation pairs had at least a 5% increase in total lipid content and at least a ~3% increase in EPA % TFAs with respect to control (Tables 21 and 22). This is a significant observation since it had previously been difficult to induce a simultaneous increase in both total lipid content and EPA % TFAs. Usually, an increase in total lipid content had corresponded with a decrease in EPA % TFAs, and vice versa.

[0247] The double mutant YlLPCAT polypeptides listed in bold and with a "+" in Tables 21 and 22, i.e., M136S_T389A, M136S_T389C, M136S_T389S, M136V_T389C, N144A_F390S, G148A_F390S, G148N_T382I and G148N_F390S, are disclosed herein as SEQ ID NOs:79, 81, 83, 85, 87, 89, 91 and 93, respectively.

EXAMPLE 7

Over-Expression of *Yarrowia lipolytica* PDAT along with Over-Expression of a Mutant *Yarrowia lipolytica* LPCAT for EPA Production

[0248] The present Example describes over-expression of a *Y. lipolytica* PDAT in a *Y. lipolytica* strain engineered to produce high levels of lipids containing eicosapentaenoic acid ["EPA"], wherein the strain also over-expresses a mutant *Y. lipolytica* LPCAT comprising a single mutation within Motif I and/or a single mutation within Motif II.

[0249] More specifically, any of the preferred mutant YlLPCAT polynucleotides described in Example 6 would be cloned into expression plasmid pY301 (SEQ ID NO:44, Example 2), to replace the polynucleotide encoding wild type YlLPCAT with a polynucleotide encoding a mutant YlLPCAT. This modified plasmid would then be used to transform any preferred strain of *Y. lipolytica* that had been engineered to produce a PUFA, e.g., EPA. The transformed *Yarrowia* would be grown and analyzed for lipid content and PUFA production as in Example 2.

SEQUENCE LISTING

[0250]

<110> E.I. du Pont de Nemours & Company, Inc.
Yadav, Narendra
Bostick, Michael
Zhang, Hongxiang
Zhu, Quinn

<120> IMPROVED PRODUCTION OF POLYUNSATURATED FATTY ACIDS BY COEXPRESSION OF ACYL-CoA:LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASES AND PHOSPHOLIPID:DIACYLGLYCEROL ACYLTRANSFERASES

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<151> 2012-06-19

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            260                 265                 270

ttc aga atc cat tac tta ttc ttg ctt ggt ttc atc cat aga ttc aag        864
Phe Arg Ile His Tyr Leu Phe Leu Leu Gly Phe Ile His Arg Phe Lys
        275                 280                 285

tac tac gct gcc tgg act att tcg gaa gga tct tgt att ttg tgc ggt        912
Tyr Tyr Ala Ala Trp Thr Ile Ser Glu Gly Ser Cys Ile Leu Cys Gly
    290                 295                 300

ttg ggt tat aat ggt tat gat tca aag aca caa aag atc aga tgg gat        960
Leu Gly Tyr Asn Gly Tyr Asp Ser Lys Thr Gln Lys Ile Arg Trp Asp
305                 310                 315                 320

cgt gtc aga aat att gac att tgg acc gta gaa acg gcg cag aat acg       1008
Arg Val Arg Asn Ile Asp Ile Trp Thr Val Glu Thr Ala Gln Asn Thr
                325                 330                 335

cgt gaa atg ttg gaa gca tgg aat atg aat act aac aag tgg cta aaa       1056
Arg Glu Met Leu Glu Ala Trp Asn Met Asn Thr Asn Lys Trp Leu Lys
            340                 345                 350

tac tct gtt tat tta cgt gtc aca aag aag ggc aaa aaa cct ggt ttc       1104
Tyr Ser Val Tyr Leu Arg Val Thr Lys Lys Gly Lys Lys Pro Gly Phe
        355                 360                 365

cgc tca act ttg ttt act ttc cta act tcc gca ttt tgg cat ggt acc       1152
Arg Ser Thr Leu Phe Thr Phe Leu Thr Ser Ala Phe Trp His Gly Thr
    370                 375                 380

aga cct ggg tac tat ctg act ttt gcg aca ggg gct ttg tac caa aca       1200
Arg Pro Gly Tyr Tyr Leu Thr Phe Ala Thr Gly Ala Leu Tyr Gln Thr
385                 390                 395                 400

tgt ggt aaa atc tac aga cgc aat ttt aga cca att ttc ttg cga gaa       1248
Cys Gly Lys Ile Tyr Arg Arg Asn Phe Arg Pro Ile Phe Leu Arg Glu
                405                 410                 415

gat ggt gtc act cct ttg cct tct aaa aaa atc tac gat tta gtt ggc       1296
Asp Gly Val Thr Pro Leu Pro Ser Lys Lys Ile Tyr Asp Leu Val Gly
            420                 425                 430

ata tat gca att aaa cta gca ttt ggt tac atg gtg caa cca ttt att       1344
Ile Tyr Ala Ile Lys Leu Ala Phe Gly Tyr Met Val Gln Pro Phe Ile
        435                 440                 445

atc ctt gat ttg aag cca tct tta atg gta tgg ggc tct gtt tat ttc       1392
Ile Leu Asp Leu Lys Pro Ser Leu Met Val Trp Gly Ser Val Tyr Phe
    450                 455                 460

tat gtt cat att att gtt gct ttc tca ttt ttc cta ttc aga gga cca       1440
Tyr Val His Ile Ile Val Ala Phe Ser Phe Phe Leu Phe Arg Gly Pro
465                 470                 475                 480

tat gct aaa caa gtt act gaa ttt ttt aaa tcc aaa caa cct aaa gaa       1488
Tyr Ala Lys Gln Val Thr Glu Phe Phe Lys Ser Lys Gln Pro Lys Glu
                485                 490                 495

ata ttc att aga aaa caa aag aag ttg gaa aaa gat att tct gca agc       1536
Ile Phe Ile Arg Lys Gln Lys Lys Leu Glu Lys Asp Ile Ser Ala Ser
            500                 505                 510

tct cca aac ttg ggt ggt ata ttg aag gca aag att gaa cat gaa aag       1584

Ser Pro Asn Leu Gly Gly Ile Leu Lys Ala Lys Ile Glu His Glu Lys
        515                 520                 525

gga aag aca gca gaa gaa gaa gaa atg aac tta ggt att cca cca att       1632
Gly Lys Thr Ala Glu Glu Glu Glu Met Asn Leu Gly Ile Pro Pro Ile
    530                 535                 540

gag tta gaa aag tgg gac aat gct aag gaa gat tgg gaa gat ttc tgc       1680
Glu Leu Glu Lys Trp Asp Asn Ala Lys Glu Asp Trp Glu Asp Phe Cys
545                 550                 555                 560

aaa gat tac aaa gaa tgg aga aat aaa aat ggt ctt gaa ata gaa gag       1728
Lys Asp Tyr Lys Glu Trp Arg Asn Lys Asn Gly Leu Glu Ile Glu Glu
                565                 570                 575

gaa aac ctt tct aaa gct ttt gaa aga ttc aag cag gaa ttt tct aac       1776
Glu Asn Leu Ser Lys Ala Phe Glu Arg Phe Lys Gln Glu Phe Ser Asn
            580                 585                 590

gct gca agt gga tca ggt gaa cgt gtg aga aaa atg agt ttt agt ggt       1824
Ala Ala Ser Gly Ser Gly Glu Arg Val Arg Lys Met Ser Phe Ser Gly
        595                 600                 605

tac tca cca aag cct att tca aaa aag gaa gag tag                       1860
Tyr Ser Pro Lys Pro Ile Ser Lys Lys Glu Glu
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<213> Saccharomyces cerevisiae

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            20                  25                  30

Phe Pro Leu Asn Ala Ile Leu Lys Arg Ile Pro Glu Lys Arg Ile Gly
        35                  40                  45

Leu Lys Cys Cys Phe Ile Ile Ser Met Ser Met Phe Tyr Leu Phe Gly
    50                  55                  60

Val Leu Asn Leu Val Ser Gly Phe Arg Thr Leu Phe Ile Ser Thr Met
65                  70                  75                  80

Phe Thr Tyr Leu Ile Ser Arg Phe Tyr Arg Ser Lys Phe Met Pro His
                85                  90                  95

Leu Asn Phe Met Phe Val Met Gly His Leu Ala Ile Asn His Ile His
            100                 105                 110

Ala Gln Phe Leu Asn Glu Gln Thr Gln Thr Thr Val Asp Ile Thr Ser
        115                 120                 125

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

Tyr Asp Gly Ser Cys Thr Ser Glu Ser Asp Phe Lys Asp Leu Thr Glu
145                 150                 155                 160

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

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

Asp Tyr Ala Asp Phe Asp Ser Trp Leu Asn Cys Glu Met Phe Arg Asp
        195                 200                 205

Leu Pro Glu Ser Lys Lys Pro Met Arg Arg His His Pro Gly Glu Arg
    210                 215                 220

Arg Gln Ile Pro Lys Asn Gly Lys Leu Ala Leu Trp Lys Val Val Gln
225                 230                 235                 240

Gly Leu Ala Trp Met Ile Leu Ser Thr Leu Gly Met Lys His Phe Pro
                245                 250                 255

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

Phe Arg Ile His Tyr Leu Phe Leu Leu Gly Phe Ile His Arg Phe Lys
        275                 280                 285

Tyr Tyr Ala Ala Trp Thr Ile Ser Glu Gly Ser Cys Ile Leu Cys Gly
    290                 295                 300

Leu Gly Tyr Asn Gly Tyr Asp Ser Lys Thr Gln Lys Ile Arg Trp Asp
305                 310                 315                 320

Arg Val Arg Asn Ile Asp Ile Trp Thr Val Glu Thr Ala Gln Asn Thr
                325                 330                 335

Arg Glu Met Leu Glu Ala Trp Asn Met Asn Thr Asn Lys Trp Leu Lys
            340                 345                 350

Tyr Ser Val Tyr Leu Arg Val Thr Lys Lys Gly Lys Lys Pro Gly Phe
        355                 360                 365

Arg Ser Thr Leu Phe Thr Phe Leu Thr Ser Ala Phe Trp His Gly Thr
    370                 375                 380

Arg Pro Gly Tyr Tyr Leu Thr Phe Ala Thr Gly Ala Leu Tyr Gln Thr
385                 390                 395                 400

Cys Gly Lys Ile Tyr Arg Arg Asn Phe Arg Pro Ile Phe Leu Arg Glu
                405                 410                 415

Asp Gly Val Thr Pro Leu Pro Ser Lys Lys Ile Tyr Asp Leu Val Gly
            420                 425                 430

Ile Tyr Ala Ile Lys Leu Ala Phe Gly Tyr Met Val Gln Pro Phe Ile
        435                 440                 445
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        435                 440                 445

Ile Leu Asp Leu Lys Pro Ser Leu Met Val Trp Gly Ser Val Tyr Phe
    450                 455                 460

Tyr Val His Ile Ile Val Ala Phe Ser Phe Phe Leu Phe Arg Gly Pro
465                 470                 475                 480

Tyr Ala Lys Gln Val Thr Glu Phe Phe Lys Ser Lys Gln Pro Lys Glu
                485                 490                 495

Ile Phe Ile Arg Lys Gln Lys Lys Leu Glu Lys Asp Ile Ser Ala Ser
            500                 505                 510

Ser Pro Asn Leu Gly Gly Ile Leu Lys Ala Lys Ile Glu His Glu Lys
        515                 520                 525

Gly Lys Thr Ala Glu Glu Glu Glu Met Asn Leu Gly Ile Pro Pro Ile
    530                 535                 540

Glu Leu Glu Lys Trp Asp Asn Ala Lys Glu Asp Trp Glu Asp Phe Cys
545                 550                 555                 560

Lys Asp Tyr Lys Glu Trp Arg Asn Lys Asn Gly Leu Glu Ile Glu Glu
                565                 570                 575

Glu Asn Leu Ser Lys Ala Phe Glu Arg Phe Lys Gln Glu Phe Ser Asn
            580                 585                 590

Ala Ala Ser Gly Ser Gly Glu Arg Val Arg Lys Met Ser Phe Ser Gly
        595                 600                 605

Tyr Ser Pro Lys Pro Ile Ser Lys Lys Glu Glu
    610                 615
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<212> DNA

<213> Yarrowia lipolytica

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<222> (1)..(1539)

<223> GenBank Accession No. XP_505624; "YALI0F19514p"

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1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg          96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa         144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg         192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca         240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg         288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt         336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc         384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt atg aag cta tcg tct ttt gga tgg aac         432
Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat gga tgg cag att gag aag ggt gag cag ctc agc gag ttc         480
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta         528
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Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc        720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc        768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cat ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc        960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat       1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc       1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct       1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc acc ttt gtg acc gct gcc atg tac cag tct gtt ggt       1200
Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga       1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc       1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta       1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac       1392
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510

taa                                                                  1539
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<210> 4

<211> 512

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<213> Yarrowia lipolytica

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe

145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430
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Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
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<301> Hideo Shindou, Miki Etoa, Ryo Morimotoa and Takao Shimizua
<302> Identification of membrane O-acyltransferase family motifs
<303> Biochemical and Biophysical Research Communications
<304> 383
<305> 3
<306> 320-325
<307> 2009-04-08
<313> (1) .. (12)

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<301> Hideo Shindou, Miki Etoa, Ryo Morimotoa and Takao Shimizua
<302> Identification of membrane O-acyltransferase family motifs
<303> Biochemical and Biophysical Research Communications
<304> 383
<305> 3
<306> 320-325

<307> 2009-04-08
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<400> 6
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1 5

<210> 7
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<301> Hideo Shindou, Miki Etoa, Ryo Morimotoa and Takao Shimizua
<302> Identification of membrane O-acyltransferase family motifs
<303> Biochemical and Biophysical Research Communications
<304> 383
<305> 3
<306> 320-325
<307> 2009-04-08
<313> (1)..(9)

<400> 7
Tyr Xaa Xaa Xaa Tyr Phe Xaa Xaa His
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<212> PRT
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<223> Xaa = Val [V] or Ile [I]

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<221> MISC_FEATURE
<222> (3)..(3)
<223> Xaa = Leu [L] or Ile [I]

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<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent Publication No. 2008-0145867-A1
<311> 2007-06-15
<312> 2008-06-19
<313> (1)..(15)

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Gly

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<223> Xaa = Ala [A] or Gly [G]

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<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent Publication No. 2008-0145867-A1
<311> 2007-06-15
<312> 2008-06-19
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1 5 10 15

Xaa Xaa Gly Xaa Gly Xaa Xaa Gly
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<210> 10
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<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent Publication No. 2008-0145867-A1
<311> 2007-06-15
<312> 2008-06-19
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1 5 10 15

Xaa Xaa Xaa Trp
20

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<223> Xaa can be any naturally occurring amino acid

<220>
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<220>
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<222> (14)..(14)
<223> Xaa = Thr [T] or Phe [F]

<300>
<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent Publication No. 2008-0145867-A1
<311> 2007-06-15
<312> 2008-06-19
<313> (1)..(15)

<400> 11
Ser Ala Xaa Trp His Gly Xaa Xaa Pro Gly Tyr Xaa Xaa Xaa Phe
1 5 10 15

<210> 12
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> membrane bound O-acyltransferase motif

<220>
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<223> Xaa = Val [V] or Ile [I]

<220>
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<223> Xaa = Leu [L] or Ile [I] or Val [V]

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<223> Xaa = Ala [A] or Cys [C] or Thr [T] or Val [V]

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<223> Xaa = Asn [N] or Asp [D] or Glu [E] or Gln [Q] or Ser [S]

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<223> Xaa = Ile [I] or Leu [L] or Tyr [Y] or Val [V]

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<223> Xaa = Ala [A] or Asn [N] or His [H] or Ser [S] or Thr [T] or Tyr [Y]

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<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent 7,732,155
<311> 2007-06-15
<312> 2010-06-08
<313> (1)..(17)

<400> 12
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1               5                  10                  15

Gly
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<210> 13
<211> 24
<212> PRT
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<220>
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<220>
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<222> (6)..(6)
<223> Xaa = Ala [A] or Gly [G] or His [H] or Phe [F] or Ser [S]

<220>
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<223> Xaa = Ala [A] or Cys [C] or Ile [I] or Val [V]

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<222> (9)..(9)
<223> Xaa = Glu [E] or Leu [L] or Met [M] or Ser [S] or Thr [T] or Tyr [Y]

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<222> (10)..(10)
<223> Xaa = Asn [N] or Ile [I] or Leu [L]

<220>
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<223> Xaa = Ala [A] or Ser [S] or Thr [T]

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<222> (13)..(13)
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<223> Xaa = Ala [A] or Ile [I] or Ser [S] or Val [V]

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<222> (16)..(16)
<223> Xaa = Asn [N] or Ile [I] or Val [V]

<220>
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<222> (17)..(17)
<223> Xaa = Ala [A] or Asn [N] or Cys [C] or Ile [I] or Leu [L]

<220>
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<222> (18)..(18)
<223> Xaa = Ala [A] or Cys [C] or Ile [I] or Ser [S] or Trp [W]

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<222> (22)..(22)
<223> Xaa = Phe [F] or Tyr [Y]

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<223> Xaa = Arg [R] or Asn [N] or Glu [E] or Lys [K] or Ser [S] or Thr [T]

<300>
<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent 7,732,155
<311> 2007-06-15
<312> 2010-06-08
<313> (1)..(15)

<400> 13

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Arg Xaa Lys Tyr Tyr Xaa Xaa Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10                  15

Xaa Xaa Gly Xaa Gly Xaa Xaa Gly
            20
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<210> 14
<211> 20
<212> PRT
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<220>
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<223> Xaa = Leu [L] or Met [M] or Phe [F] or Thr [T]

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<220>
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<222> (4)..(4)
<223> Xaa = Asp [D] or Gln [Q] or Lys [K] or Pro [P] or Thr [T]

<220>
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<222> (5)..(5)
<223> Xaa = Asn [N] or Ser [S]

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<223> Xaa = Asp [D] or Cys [C] or Gly [G] or Glu [E] or Gln [Q] or Met [M] or Thr [T]

<220>
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<223> Xaa = Ala [A] or Ile [I] or Leu [L] or Met [M] or Phe [F] or Tyr [Y]

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<222> (12)..(12)
<223> Xaa = Ala [A] or Asn [N] or Met [M] or Phe [F] or Ser [S] or Val [V]

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<223> Xaa = Cys [C] or Ile [I] or Lys [K] or Met [M]

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<300>
<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent 7,732,155
<311> 2007-06-15
<312> 2010-06-08
<313> (1)..(15)

<400> 14

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Xaa Xaa Xaa Trp
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<210> 15

<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<223> Xaa = Leu [L] or Phe [F] or Thr [T] or Val [V]

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<223> Xaa = Arg [R] or Ser [S] or Tyr [Y]

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<223> Xaa = Ile [I] or Met [M] or Tyr [Y]

<220>
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<223> Xaa = Ile [I] or Leu [L] or Met [M] or Phe [F]

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<223> Xaa = Thr [T] or Phe [F]

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<302> GENES ENCODING A NOVEL TYPE OF LYSOPHOPHATIDYLCHOLINE ACYLTRANSFERASES AND THEIR USE TO INCREASE TRIACYLGLYCEROL PRODUCTION AND/OR MODIFY FATTY ACID COMPOSITION

<310> U.S. Patent 7,732,155
<311> 2007-06-15
<312> 2010-06-08
<313> (1)..(15)

<400> 15

Ser	Ala	Xaa	Trp	His	Gly	Xaa	Xaa	Pro	Gly	Tyr	Xaa	Xaa	Xaa	Phe
1				5				10					15	

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<223> Xaa = Val [V] or Ile [I]

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<223> Xaa can be any naturally occurring amino acid

<300>
<302> IMPROVEMENT OF LONG CHAIN OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACID BIOSYNTHESIS BY EXPRESSION OF ACYL-CoA LYSOPHOSPHOLIPID ACYLTRANSFERASES

<310> U.S. Pat. Pub. No. 2010-0317882-A1
<311> 2010-06-14
<312> 2010-12-16
<313> (1)..(7)

<400> 16
Met Xaa Leu Xaa Xaa Lys Leu
1 5

<210> 17
<211> 8
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<220>
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<220>
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<223> Xaa can be any naturally occurring amino acid

<300>
<302> IMPROVEMENT OF LONG CHAIN OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACID BIOSYNTHESIS BY EXPRESSION OF ACYL-CoA LYSOPHOSPHOLIPID ACYLTRANSFERASES

<310> U.S. Pat. Pub. No. 2010-0317882-A1
<311> 2010-06-14
<312> 2010-12-16
<313> (1)..(8)

<400> 17
Arg Xaa Lys Tyr Tyr Xaa Xaa Trp
1 5

<210> 18
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<300>
<302> IMPROVEMENT OF LONG CHAIN OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACID BIOSYNTHESIS BY EXPRESSION OF ACYL-CoA LYSOPHOSPHOLIPID ACYLTRANSFERASES

<310> U.S. Pat. Pub. No. 2010-0317882-A1
<311> 2010-06-14
<312> 2010-12-16
<313> (1)..(6)

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Ser Ala Xaa Trp His Gly

1 5

<210> 19
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<213> Yarrowia lipolytica

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<223> Xaa can be any naturally occurring amino acid

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    130                 135                 140

Xaa Xaa Xaa Xaa Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220
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Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Xaa Trp His Gly Xaa Xaa Pro
    370                 375                 380

Xaa Tyr Tyr Xaa Xaa Xaa Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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<210> 20
<211> 945
<212> DNA
<213> Mortierella alpina

<220>
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<222> (1)..(945)
<223> LPAAT1

<300>
<302> High eicosapentaenoic acid producing strains of Yarrowia lipolytica

<310> U.S. 7,879,591
<311> 2009-06-19
<312> 2011-02-01
<313> (1)..(945)

<300>
<302> High eicosapentaenoic acid producing strains of Yarrowia lipolytica

<310> WO 2006/052870
<311> 2005-11-03
<312> 2006-05-18
<313> (1)..(945)

<400> 20

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Met Ser Ile Gly Ser Ser Asn Pro Val Leu Leu Ala Ala Ile Pro Phe
1               5                   10                  15

gtc tac ctc ttc gtc ctc cct cgt gtc ctc gcc ttc ctc cct caa aag         96
Val Tyr Leu Phe Val Leu Pro Arg Val Leu Ala Phe Leu Pro Gln Lys
            20                  25                  30

gcc cag ttc ctc gca aaa tgc atc gtg gtc ttg atc gcc acc ctt atc        144
Ala Gln Phe Leu Ala Lys Cys Ile Val Val Leu Ile Ala Thr Leu Ile
        35                  40                  45

atg tcc gtc gca ggc tgc ttc att tcc atc gtc tgt gcg ctc ctc gat        192
Met Ser Val Ala Gly Cys Phe Ile Ser Ile Val Cys Ala Leu Leu Asp
    50                  55                  60

aaa cgc tat gtg atc aac tac gtc gtc tca aga ctc ttc tca ttc ctc        240
Lys Arg Tyr Val Ile Asn Tyr Val Val Ser Arg Leu Phe Ser Phe Leu
65                  70                  75                  80

gct gca aga ccc tgc ggt gtc acc tac aag atc gtc ggc gag gaa cat        288
Ala Ala Arg Pro Cys Gly Val Thr Tyr Lys Ile Val Gly Glu Glu His
                85                  90                  95

ctg gac aag tac ccc gcc att gtc gtc tgc aac cac cag agc tcc atg        336
Leu Asp Lys Tyr Pro Ala Ile Val Val Cys Asn His Gln Ser Ser Met
            100                 105                 110

gac atg atg gtc ctg gga cgc gtc ttc cca aag cac tgt gtc gtc atg        384
Asp Met Met Val Leu Gly Arg Val Phe Pro Lys His Cys Val Val Met
        115                 120                 125

gca aag aag gaa ctt ctt tac ttt ccg ttc ctg ggc atg ttt atg aag        432
Ala Lys Lys Glu Leu Leu Tyr Phe Pro Phe Leu Gly Met Phe Met Lys
    130                 135                 140

ctg agt aac gcc atc ttc att gac cgc aag aac cac aag aag gcg atc        480
Leu Ser Asn Ala Ile Phe Ile Asp Arg Lys Asn His Lys Lys Ala Ile
145                 150                 155                 160

gag tcc acc acc caa gct gtc gcc gac atg aag aag cac aac tct gga        528
Glu Ser Thr Thr Gln Ala Val Ala Asp Met Lys Lys His Asn Ser Gly
                165                 170                 175

atc tgg att ttc ccc gaa gga aca cgt tcc cgc ttg gac aag gcc gat        576
Ile Trp Ile Phe Pro Glu Gly Thr Arg Ser Arg Leu Asp Lys Ala Asp
            180                 185                 190

ctc ttg ccc ttc aag aag gga gcc ttc cac ctc gcc att caa gcc caa        624
Leu Leu Pro Phe Lys Lys Gly Ala Phe His Leu Ala Ile Gln Ala Gln
        195                 200                 205

ctc ccg atc ctc ccc atc atc tcg caa gga tac tca cac atc tac gat        672
Leu Pro Ile Leu Pro Ile Ile Ser Gln Gly Tyr Ser His Ile Tyr Asp
    210                 215                 220

tcg tca aaa cgc tac ttc ccc ggt gga gag ctc gag atc aga gtc ctg        720
Ser Ser Lys Arg Tyr Phe Pro Gly Gly Glu Leu Glu Ile Arg Val Leu
225                 230                 235                 240

gaa cct atc ccc acc acg gga ttg acc aca gac gat gtg aac gac ctg        768
Glu Pro Ile Pro Thr Thr Gly Leu Thr Thr Asp Asp Val Asn Asp Leu
                245                 250                 255

atg gac aag act cgc aac ctg atg ctg aag cac ctc aag gag atg gat        816
Met Asp Lys Thr Arg Asn Leu Met Leu Lys His Leu Lys Glu Met Asp
            260                 265                 270

tct caa tac tcc tcc tcc acc gct gaa aac gga tcg acc cat att gac        864
Ser Gln Tyr Ser Ser Ser Thr Ala Glu Asn Gly Ser Thr His Ile Asp
        275                 280                 285

gcc gat atc gca aag tca act gcc aca tcg atc gga aac acg gac gat        912
Ala Asp Ile Ala Lys Ser Thr Ala Thr Ser Ile Gly Asn Thr Asp Asp
    290                 295                 300

gct atc aca aag agg agg aca cca aaa gag tag                            945
Ala Ile Thr Lys Arg Arg Thr Pro Lys Glu
305                 310
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<210> 21
<211> 314
<212> PRT
<213> Mortierella alpina

<400> 21

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1               5                   10                  15
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            20                  25                  30

Ala Gln Phe Leu Ala Lys Cys Ile Val Val Leu Ile Ala Thr Leu Ile
        35                  40                  45

Met Ser Val Ala Gly Cys Phe Ile Ser Ile Val Cys Ala Leu Leu Asp
    50                  55                  60

Lys Arg Tyr Val Ile Asn Tyr Val Val Ser Arg Leu Phe Ser Phe Leu
65                  70                  75                  80

Ala Ala Arg Pro Cys Gly Val Thr Tyr Lys Ile Val Gly Glu Glu His
                85                  90                  95

Leu Asp Lys Tyr Pro Ala Ile Val Val Cys Asn His Gln Ser Ser Met
            100                 105                 110

Asp Met Met Val Leu Gly Arg Val Phe Pro Lys His Cys Val Val Met
        115                 120                 125

Ala Lys Lys Glu Leu Leu Tyr Phe Pro Phe Leu Gly Met Phe Met Lys
    130                 135                 140

Leu Ser Asn Ala Ile Phe Ile Asp Arg Lys Asn His Lys Lys Ala Ile
145                 150                 155                 160

Glu Ser Thr Thr Gln Ala Val Ala Asp Met Lys Lys His Asn Ser Gly
                165                 170                 175

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

Leu Leu Pro Phe Lys Lys Gly Ala Phe His Leu Ala Ile Gln Ala Gln
        195                 200                 205

Leu Pro Ile Leu Pro Ile Ile Ser Gln Gly Tyr Ser His Ile Tyr Asp
    210                 215                 220

Ser Ser Lys Arg Tyr Phe Pro Gly Gly Glu Leu Glu Ile Arg Val Leu
225                 230                 235                 240

Glu Pro Ile Pro Thr Thr Gly Leu Thr Thr Asp Asp Val Asn Asp Leu
                245                 250                 255

Met Asp Lys Thr Arg Asn Leu Met Leu Lys His Leu Lys Glu Met Asp
            260                 265                 270

Ser Gln Tyr Ser Ser Ser Thr Ala Glu Asn Gly Ser Thr His Ile Asp
        275                 280                 285

Ala Asp Ile Ala Lys Ser Thr Ala Thr Ser Ile Gly Asn Thr Asp Asp
    290                 295                 300

Ala Ile Thr Lys Arg Arg Thr Pro Lys Glu
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<210> 22

<211> 1549

<212> DNA

<213> Yarrowia lipolytica

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<221> CDS

<222> (501)..(1349)

<223> LPAAT1

<300>

<302> High eicosapentaenoic acid producing strains of Yarrowia lipolytica

<310> U.S. 7,932,077

<311> 2005-11-02

<312> 2011-04-26

<313> (1)..(1549)

<300>
<302> High eicosapentaenoic acid producing strains of Yarrowia lipolytica

<310> WO 2006/052870
<311> 2005-11-03
<312> 2006-05-18
<313> (1)..(1549)

<400> 22

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tgcacaaggg acttcaagag gggccaattt catgcggaca catggcgcaa aaaacgcccg      120

actttgatta cacagacacg taataacgac gaagccgaga tgagcacacg tggccaagtc      180

tgccaatggc cccctggacc cccctgacaa agtttcccaa caagcccagc cgtgcatggt      240

gtgtttttgt gcggagacac acgccaatta ggctcatttg agggtatgca gcgaaaaaaa      300

attagtgtgg gtagtttgtt tgcaggaatc aagtgggtgg ttgaaaaaca agaaagagcg      360

acgacaagag agagagaaaa agagagagag actccataaa gcgtgcatca aaattaaggt      420

gtgtgactat ccgaaaacca aacatgaaca gttggatata tgtcgctgtg attgcagttg      480

ctgccgttct cattgcccga atg tcc gtt gca tcc aag ctc gtc ttc tac gtc      533
                    Met Ser Val Ala Ser Lys Leu Val Phe Tyr Val
                    1               5                   10

cgc gcc gcc atc gcc gtg gtc atc ttt gcc gcc tgt gcc acc tac ggc       581
Arg Ala Ala Ile Ala Val Val Ile Phe Ala Ala Cys Ala Thr Tyr Gly
            15                  20                  25

gtg ctg gcg tcc acc att ctc acc gcc atc ggc aag cag ggc ctg gcc       629
Val Leu Ala Ser Thr Ile Leu Thr Ala Ile Gly Lys Gln Gly Leu Ala
        30                  35                  40

caa tgg acc gtt gcc aga gcc ttc tac tac tcg gtg cgc atc ttc ctg       677
Gln Trp Thr Val Ala Arg Ala Phe Tyr Tyr Ser Val Arg Ile Phe Leu
    45                  50                  55

ggt atc agc atc aag ctg cgt agc cgg cag gtg acc gga acc gcc ggt       725
Gly Ile Ser Ile Lys Leu Arg Ser Arg Gln Val Thr Gly Thr Ala Gly
60                  65                  70                  75

ctg gat gcc tcc aag atc cag gtc gcc aac acc acc aag ccc att gac       773
Leu Asp Ala Ser Lys Ile Gln Val Ala Asn Thr Thr Lys Pro Ile Asp
                80                  85                  90

gac atc acc aaa cac ctg ccc cga cca tgc att ctg att tcc aac cac       821
Asp Ile Thr Lys His Leu Pro Arg Pro Cys Ile Leu Ile Ser Asn His
            95                  100                 105

cag aac gaa atg gac att ctg gtg ctc ggt cgc atc ttc ccc cag tac       869
Gln Asn Glu Met Asp Ile Leu Val Leu Gly Arg Ile Phe Pro Gln Tyr
        110                 115                 120

tgc tcc gtc acc gcc aaa aag gcc ctc aag tgg tac cct ctg ctg ggc       917
Cys Ser Val Thr Ala Lys Lys Ala Leu Lys Trp Tyr Pro Leu Leu Gly
    125                 130                 135

cag ttc atg gcg ctg tcc ggc acc atc ttc ctg gac cga aag gac cga       965
Gln Phe Met Ala Leu Ser Gly Thr Ile Phe Leu Asp Arg Lys Asp Arg
140                 145                 150                 155

acc aag tcc gtg cag acc ctc ggc ggc gcc gtc aag acc atc cag agc      1013

Thr Lys Ser Val Gln Thr Leu Gly Gly Ala Val Lys Thr Ile Gln Ser
                160                 165                 170

ggc aac gga ggc aag ggc cag agc gtc ttc atg ttc ccc gag gga acc      1061
Gly Asn Gly Gly Lys Gly Gln Ser Val Phe Met Phe Pro Glu Gly Thr
            175                 180                 185

cga tcc tac tcc aag gac gtc ggc atc atg ccc ttc aag aag ggc tgt      1109
Arg Ser Tyr Ser Lys Asp Val Gly Ile Met Pro Phe Lys Lys Gly Cys
        190                 195                 200

ttc cac ctg gcg gtc cag tcg ggc gct ccc att gtc ccc gtg gtg gtc      1157
Phe His Leu Ala Val Gln Ser Gly Ala Pro Ile Val Pro Val Val Val
    205                 210                 215

cag aac acc tcc cga atg ttt tct ttc ggc cga ggc aag ctg gac gcc      1205
Gln Asn Thr Ser Arg Met Phe Ser Phe Gly Arg Gly Lys Leu Asp Ala
220                 225                 230                 235

gga gag atc ctt gtc gac gtc ctg agc ccc att gag acc aag ggt ctg      1253
Gly Glu Ile Leu Val Asp Val Leu Ser Pro Ile Glu Thr Lys Gly Leu
                240                 245                 250

gac gcc agc aac gtc gac gct ctc atg gcc acc act tat aag gcc atg      1301
Asp Ala Ser Asn Val Asp Ala Leu Met Ala Thr Thr Tyr Lys Ala Met
            255                 260                 265
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tgc gag act gcc gac cag att ggc tac gct ggc cag aag act cag tag       1349
Cys Glu Thr Ala Asp Gln Ile Gly Tyr Ala Gly Gln Lys Thr Gln
        270                 275                 280

agactgcagc acaagaagtg cttgtagcta ctttaggaga gagataggta atatgaaaca     1409

tttttcagat cgacacccac ggcgaaccat tggctgtgga gctatgggtg aatggattaa     1469

tatagcaacg aaatctacct cgattaccaa cgcaaaacga gcccactttc tctgtactgt     1529

gctatatcgt gtatacccca                                                 1549
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<210> 23

<211> 282

<212> PRT

<213> Yarrowia lipolytica

<400> 23

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Val Val Ile Phe Ala Ala Cys Ala Thr Tyr Gly Val Leu Ala Ser Thr
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Ile Leu Thr Ala Ile Gly Lys Gln Gly Leu Ala Gln Trp Thr Val Ala
        35                  40                  45

Arg Ala Phe Tyr Tyr Ser Val Arg Ile Phe Leu Gly Ile Ser Ile Lys
    50                  55                  60

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

Ile Gln Val Ala Asn Thr Thr Lys Pro Ile Asp Asp Ile Thr Lys His
                85                  90                  95

Leu Pro Arg Pro Cys Ile Leu Ile Ser Asn His Gln Asn Glu Met Asp
            100                 105                 110

Ile Leu Val Leu Gly Arg Ile Phe Pro Gln Tyr Cys Ser Val Thr Ala
        115                 120                 125

Lys Lys Ala Leu Lys Trp Tyr Pro Leu Leu Gly Gln Phe Met Ala Leu
    130                 135                 140

Ser Gly Thr Ile Phe Leu Asp Arg Lys Asp Arg Thr Lys Ser Val Gln
145                 150                 155                 160

Thr Leu Gly Gly Ala Val Lys Thr Ile Gln Ser Gly Asn Gly Gly Lys
                165                 170                 175

Gly Gln Ser Val Phe Met Phe Pro Glu Gly Thr Arg Ser Tyr Ser Lys
            180                 185                 190

Asp Val Gly Ile Met Pro Phe Lys Lys Gly Cys Phe His Leu Ala Val
        195                 200                 205

Gln Ser Gly Ala Pro Ile Val Pro Val Val Val Gln Asn Thr Ser Arg
    210                 215                 220

Met Phe Ser Phe Gly Arg Gly Lys Leu Asp Ala Gly Glu Ile Leu Val
225                 230                 235                 240

Asp Val Leu Ser Pro Ile Glu Thr Lys Gly Leu Asp Ala Ser Asn Val
                245                 250                 255

Asp Ala Leu Met Ala Thr Thr Tyr Lys Ala Met Cys Glu Thr Ala Asp
            260                 265                 270

Gln Ile Gly Tyr Ala Gly Gln Lys Thr Gln
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<210> 24

<211> 303

<212> PRT

<213> Saccharomyces cerevisiae

<220>

<221> MISC_FEATURE

<222> (1)..(303)

<223> Slc1p; GenBank Accession No. NP_010231

<400> 24

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Val Leu Ala Leu Ala Gly Cys Gly Phe Tyr Gly Val Ile Ala Ser Ile
            20                  25                  30

Leu Cys Thr Leu Ile Gly Lys Gln His Leu Ala Gln Trp Ile Thr Ala
        35                  40                  45

Arg Cys Phe Tyr His Val Met Lys Leu Met Leu Gly Leu Asp Val Lys
    50                  55                  60

Val Val Gly Glu Glu Asn Leu Ala Lys Lys Pro Tyr Ile Met Ile Ala
65                  70                  75                  80

Asn His Gln Ser Thr Leu Asp Ile Phe Met Leu Gly Arg Ile Phe Pro
                85                  90                  95

Pro Gly Cys Thr Val Thr Ala Lys Lys Ser Leu Lys Tyr Val Pro Phe
            100                 105                 110

Leu Gly Trp Phe Met Ala Leu Ser Gly Thr Tyr Phe Leu Asp Arg Ser
        115                 120                 125

Lys Arg Gln Glu Ala Ile Asp Thr Leu Asn Lys Gly Leu Glu Asn Val
    130                 135                 140

Lys Lys Asn Lys Arg Ala Leu Trp Val Phe Pro Glu Gly Thr Arg Ser
145                 150                 155                 160

Tyr Thr Ser Glu Leu Thr Met Leu Pro Phe Lys Lys Gly Ala Phe His
                165                 170                 175

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

Thr Ser Thr Leu Val Ser Pro Lys Tyr Gly Val Phe Asn Arg Gly Cys
        195                 200                 205

Met Ile Val Arg Ile Leu Lys Pro Ile Ser Thr Glu Asn Leu Thr Lys
    210                 215                 220

Asp Lys Ile Gly Glu Phe Ala Glu Lys Val Arg Asp Gln Met Val Asp

225                 230                 235                 240

Thr Leu Lys Glu Ile Gly Tyr Ser Pro Ala Ile Asn Asp Thr Thr Leu
                245                 250                 255

Pro Pro Gln Ala Ile Glu Tyr Ala Ala Leu Gln His Asp Lys Lys Val
            260                 265                 270

Asn Lys Lys Ile Lys Asn Glu Pro Val Pro Ser Val Ser Ile Ser Asn
        275                 280                 285

Asp Val Asn Thr His Asn Glu Gly Ser Ser Val Lys Lys Met His
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<210> 25

<211> 7

<212> PRT

<213> Artificial Sequence

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<223> 1-acyl-sn-glycerol-3-phosphate acyltransferase motif

<220>

<221> misc_feature

<222> (3)..(6)

<223> Xaa can be any naturally occurring amino acid

<400> 25
Asn His Xaa Xaa Xaa Xaa Asp
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<210> 26
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> 1-acyl-sn-glycerol-3-phosphate acyltransferase motif

<400> 26
Glu Gly Thr Arg
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<210> 27
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> 1-acyl-sn-glycerol-3-phosphate acyltransferase motif

<220>
<221> misc_feature
<222> (2)..(3)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Xaa = Asp [D] or Arg [R]

<300>
<301> Tal M. Lewin, Ping Wang, and Rosalind A. Coleman
<302> Analysis of Amino Acid Motifs Diagnostic for the sn-Glycerol-3-phosphate Acyltransferase Reaction
<303> Biochemistry
<304> 38
<305> 18
<306> 57645771
<307> 1999-04-15
<313> (1)..(7)

<300>
<301> Atsushi Yamashita, Hiroki Nakanishia, Hiroshi Suzukia, Ryo Kamataa, Ken Tanakaa, Keizo Wakua and Takayuki Sugiura
<302> Topology of acyltransferase motifs and substrate specificity and accessibility in 1-acyl-sn-glycero-3-phosphate acyltransferase 1
<303> Biochimica et Biophysica Acta
<304> 1771
<305> 9
<306> 1202-1215
<307> 2007-07-17
<313> (1)..(7)

<400> 27
Gly Xaa Xaa Phe Ile Xaa Arg
1 5

<210> 28
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> 1-acyl-sn-glycerol-3-phosphate acyltransferase motif

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> Xaa = Val [V] or Ile [I]

<220>
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<222> (2)..(2)
<223> Xaa = Pro [P] or Xaa

<220>
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<222> (3)..(3)
<223> Xaa = Ile [I] or Val [V] or Leu [L]

<220>
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<222> (4)..(4)
<223> Xaa = Ile [I] or Val [V]

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Xaa = Val [V] or Ile [I]

<300>
<301> Atsushi Yamashita, Hiroki Nakanishia, Hiroshi Suzukia, Ryo Kamataa, Ken Tanakaa, Keizo Wakua and Takayuki Sugiura
<302> Topology of acyltransferase motifs and substrate specificity and accessibility in 1-acyl-sn-glycero-3-phosphate acyltransferase 1
<303> Biochimica et Biophysica Acta
<304> 1771
<305> 9
<306> 1202-1215
<307> 2007-07-17
<313> (1)..(6)

<400> 28
Xaa Xaa Xaa Xaa Pro Xaa
1 5

<210> 29
<211> 6
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<220>
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<302> Topology of acyltransferase motifs and substrate specificity and accessibility in 1-acyl-sn-glycero-3-phosphate acyltransferase 1
<303> Biochimica et Biophysica Acta
<304> 1771
<305> 9
<306> 1202-1215
<307> 2007-07-17
<313> (1)..(6)

<400> 29
Ile Val Pro Ile Val Met
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<210> 30
<211> 661
<212> PRT

<213> Saccharomyces cerevisiae

<220>
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<222> (1)..(661)
<223> GenBank Accession No. P40345

<400> 30

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Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
            20                  25                  30

Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
        35                  40                  45

Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
    50                  55                  60

Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65                  70                  75                  80

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

Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
            100                 105                 110

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

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

Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145                 150                 155                 160

Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
                165                 170                 175

Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
            180                 185                 190

Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
        195                 200                 205

Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
    210                 215                 220

Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225                 230                 235                 240

Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
                245                 250                 255

Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
            260                 265                 270

Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
        275                 280                 285

Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
    290                 295                 300

Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305                 310                 315                 320

Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
                325                 330                 335

Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
            340                 345                 350

Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
        355                 360                 365
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Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
    370                 375                 380

Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385                 390                 395                 400

Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
                405                 410                 415

Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
            420                 425                 430

Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
        435                 440                 445

Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
    450                 455                 460

Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465                 470                 475                 480

Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
                485                 490                 495

Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
            500                 505                 510

Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
        515                 520                 525

Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
    530                 535                 540

Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545                 550                 555                 560

Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
                565                 570                 575

Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
            580                 585                 590

Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
        595                 600                 605

Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
    610                 615                 620

Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625                 630                 635                 640

Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
                645                 650                 655

Met Pro Phe Pro Met
            660
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<210> 31
<211> 1947
<212> DNA
<213> Yarrowia lipolytica

<220>
<221> CDS
<222> (1)..(1947)
<223> phospholipid:diacylglycerol acyltransferase

<300>
<302> ACYLTRANSFERASES FOR ALTERATION OF POLYUNSATURATED FATTY ACIDS AND OIL CONTENT IN OLEAGINOUS YEASTS

<310> US 7,901,928
<311> 2009-03-11
<312> 2011-03-08
<313> (1)..(1947)

<400> 31

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

gca gtg gag gtg gct gac tcc gag tcc gag gcc aag acc gac gtc cac         96
Ala Val Glu Val Ala Asp Ser Glu Ser Glu Ala Lys Thr Asp Val His
            20                  25                  30

gtt cac cac cat cat cac cac cac aag cga aaa tcc gtc aag ggc aag        144
Val His His His His His His His Lys Arg Lys Ser Val Lys Gly Lys
        35                  40                  45

att ctc aac ttc ttc acc cga agt cga cgt atc acc ttc gtc ctc ggc        192
Ile Leu Asn Phe Phe Thr Arg Ser Arg Arg Ile Thr Phe Val Leu Gly
    50                  55                  60

gcc gtg gtc ggt gtg ata gcc gcg gga tac tac gct gcg cca ccg gag        240
Ala Val Val Gly Val Ile Ala Ala Gly Tyr Tyr Ala Ala Pro Pro Glu
65                  70                  75                  80

ctc agc att gat atc gat gct ctt ctc ggc gac ttg ccc tcg ttc gac        288
Leu Ser Ile Asp Ile Asp Ala Leu Leu Gly Asp Leu Pro Ser Phe Asp
                85                  90                  95

ttt gac gct cta tct ctc gac aac ttg tcc atg gac agt gtg tcg gac        336
Phe Asp Ala Leu Ser Leu Asp Asn Leu Ser Met Asp Ser Val Ser Asp
            100                 105                 110

ttt gta caa gac atg aaa tcg cgg ttt ccg acc aag att ctg cag gag        384
Phe Val Gln Asp Met Lys Ser Arg Phe Pro Thr Lys Ile Leu Gln Glu
        115                 120                 125

gcg gcc aag atc gag aag cac cag aaa agc gaa cag aag gct gcc cct        432
Ala Ala Lys Ile Glu Lys His Gln Lys Ser Glu Gln Lys Ala Ala Pro
    130                 135                 140

ttt gct gtg ggc aag gct atg aag agc gag gga ctc aac gcc aag tac        480
Phe Ala Val Gly Lys Ala Met Lys Ser Glu Gly Leu Asn Ala Lys Tyr
145                 150                 155                 160

ccg gtg gtg ctg gtg ccc ggc gtc atc tcc acg gga ctg gag agc tgg        528
Pro Val Val Leu Val Pro Gly Val Ile Ser Thr Gly Leu Glu Ser Trp
                165                 170                 175

tcc ctg gag gga acc gag gag tgt ccc acc gag tcg cac ttc aga aag        576
Ser Leu Glu Gly Thr Glu Glu Cys Pro Thr Glu Ser His Phe Arg Lys
            180                 185                 190

cga atg tgg ggc tcc tgg tac atg atc cga gtc atg ctg ctg gac aag        624
Arg Met Trp Gly Ser Trp Tyr Met Ile Arg Val Met Leu Leu Asp Lys
        195                 200                 205

tac tgc tgg ctg cag aac ctg atg ctg gac aca gag acc ggt cta gac        672
Tyr Cys Trp Leu Gln Asn Leu Met Leu Asp Thr Glu Thr Gly Leu Asp
    210                 215                 220

cct ccc cat ttc aag ctg cga gcc gcc cag gga ttt gcc tcc gcc gac        720
Pro Pro His Phe Lys Leu Arg Ala Ala Gln Gly Phe Ala Ser Ala Asp
225                 230                 235                 240

ttc ttt atg gca ggc tac tgg ctg tgg aac aag ctg ctc gag aac ctg        768

Phe Phe Met Ala Gly Tyr Trp Leu Trp Asn Lys Leu Leu Glu Asn Leu
                245                 250                 255

gct gtt att gga tac gat acg gat aca atg tct gct gcg gcg tac gac        816
Ala Val Ile Gly Tyr Asp Thr Asp Thr Met Ser Ala Ala Ala Tyr Asp
            260                 265                 270

tgg aga ctg tcc tac cct gat ttg gag cac cga gac gga tac ttc tcc        864
Trp Arg Leu Ser Tyr Pro Asp Leu Glu His Arg Asp Gly Tyr Phe Ser
        275                 280                 285

aag ctc aaa gct tca atc gaa gag act aag cgt atg aca ggt gag aag        912
Lys Leu Lys Ala Ser Ile Glu Glu Thr Lys Arg Met Thr Gly Glu Lys
    290                 295                 300

aca gtt ctg acg ggc cat tcc atg ggc tcc cag gtc atc ttc tac ttc        960
Thr Val Leu Thr Gly His Ser Met Gly Ser Gln Val Ile Phe Tyr Phe
305                 310                 315                 320

atg aag tgg gct gag gcc gag gga tat gga gga gga ggt ccc aac tgg       1008
Met Lys Trp Ala Glu Ala Glu Gly Tyr Gly Gly Gly Gly Pro Asn Trp
                325                 330                 335

gtc aat gac cat att gaa tcc ttt gtc gac att tcc ggc tcc atg ctg       1056
Val Asn Asp His Ile Glu Ser Phe Val Asp Ile Ser Gly Ser Met Leu
            340                 345                 350

ggt act ccc aag acc ctg gtt gct ctt ctg tct gga gaa atg aag gat       1104
Gly Thr Pro Lys Thr Leu Val Ala Leu Leu Ser Gly Glu Met Lys Asp
        355                 360                 365

acc gtg cag ctg aac gcg atg gct gtg tat gga ctg gag cag ttc ttc       1152
Thr Val Gln Leu Asn Ala Met Ala Val Tyr Gly Leu Glu Gln Phe Phe
    370                 375                 380

tct cga cga gag cga gcc gat ctg ctg cga aca tgg gga gga att gct       1200
Ser Arg Arg Glu Arg Ala Asp Leu Leu Arg Thr Trp Gly Gly Ile Ala
385                 390                 395                 400

tcc atg att ccc aag ggt ggt aag gct atc tgg ggt gat cat tct gga       1248
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Ser Met Ile Pro Lys Gly Gly Lys Ala Ile Trp Gly Asp His Ser Gly
                405                 410                 415

gcc cct gat gac gag ccc ggc cag aat gtc acc ttt ggc aac ttc atc       1296
Ala Pro Asp Asp Glu Pro Gly Gln Asn Val Thr Phe Gly Asn Phe Ile
            420                 425                 430

aag ttc aag gag tcc ttg acc gag tac tct gct aag aac ctc acc atg       1344
Lys Phe Lys Glu Ser Leu Thr Glu Tyr Ser Ala Lys Asn Leu Thr Met
        435                 440                 445

gat gaa acc gtt gac ttc ctg tat tct cag tct ccc gag tgg ttt gtg       1392
Asp Glu Thr Val Asp Phe Leu Tyr Ser Gln Ser Pro Glu Trp Phe Val
    450                 455                 460

aac cga acc gag ggt gct tac tcc ttt gga att gcc aag act cga aag       1440
Asn Arg Thr Glu Gly Ala Tyr Ser Phe Gly Ile Ala Lys Thr Arg Lys
465                 470                 475                 480

cag gtt gag cag aat gag aag cga cct tct acc tgg agc aac cct ctg       1488
Gln Val Glu Gln Asn Glu Lys Arg Pro Ser Thr Trp Ser Asn Pro Leu
                485                 490                 495

gaa gct gct ctc ccc aat gcc ccc gat ctc aag atc tac tgc ttc tat       1536
Glu Ala Ala Leu Pro Asn Ala Pro Asp Leu Lys Ile Tyr Cys Phe Tyr
            500                 505                 510

gga gtc ggt aag gat acc gag cga gcc tac tac tac cag gat gag ccc       1584
Gly Val Gly Lys Asp Thr Glu Arg Ala Tyr Tyr Tyr Gln Asp Glu Pro
        515                 520                 525

aat ccc gag cag acc aac ttg aac gtc agt atc gct gga aac gac cct       1632
Asn Pro Glu Gln Thr Asn Leu Asn Val Ser Ile Ala Gly Asn Asp Pro
    530                 535                 540

gat ggt gtg ctt atg ggt cag ggc gat gga acc gtc tcc ctt gtg acc       1680
Asp Gly Val Leu Met Gly Gln Gly Asp Gly Thr Val Ser Leu Val Thr
545                 550                 555                 560

cat acc atg tgt cac cga tgg aag gac gag aat tcc aag ttc aac cct       1728
His Thr Met Cys His Arg Trp Lys Asp Glu Asn Ser Lys Phe Asn Pro
                565                 570                 575

ggt aac gcc cag gtc aag gtt gtg gag atg ttg cac cag cct gat cga       1776
Gly Asn Ala Gln Val Lys Val Val Glu Met Leu His Gln Pro Asp Arg
            580                 585                 590

ctt gat att cga ggc ggt gct cag act gcc gag cat gtg gac att ctg       1824
Leu Asp Ile Arg Gly Gly Ala Gln Thr Ala Glu His Val Asp Ile Leu
        595                 600                 605

ggg cgt tct gag ttg aac gag atg gtt ctg aag gtg gct agt gga aag       1872
Gly Arg Ser Glu Leu Asn Glu Met Val Leu Lys Val Ala Ser Gly Lys
    610                 615                 620

gga aat gag att gaa gag aga gtc atc tcc aac att gat gag tgg gtg       1920
Gly Asn Glu Ile Glu Glu Arg Val Ile Ser Asn Ile Asp Glu Trp Val
625                 630                 635                 640

tgg aag att gat ctc ggc agc aat tag                                  1947
Trp Lys Ile Asp Leu Gly Ser Asn
                645
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<210> 32

<211> 648

<212> PRT

<213> Yarrowia lipolytica

<400> 32

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

Ala Val Glu Val Ala Asp Ser Glu Ser Glu Ala Lys Thr Asp Val His
            20                  25                  30

Val His His His His His His His Lys Arg Lys Ser Val Lys Gly Lys
        35                  40                  45

Ile Leu Asn Phe Phe Thr Arg Ser Arg Arg Ile Thr Phe Val Leu Gly
    50                  55                  60

Ala Val Val Gly Val Ile Ala Ala Gly Tyr Tyr Ala Ala Pro Pro Glu
65                  70                  75                  80

Leu Ser Ile Asp Ile Asp Ala Leu Leu Gly Asp Leu Pro Ser Phe Asp
                85                  90                  95

Phe Asp Ala Leu Ser Leu Asp Asn Leu Ser Met Asp Ser Val Ser Asp
            100                 105                 110

Phe Val Gln Asp Met Lys Ser Arg Phe Pro Thr Lys Ile Leu Gln Glu
        115                 120                 125
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```
Ala Ala Lys Ile Glu Lys His Gln Lys Ser Glu Gln Lys Ala Ala Pro
    130                 135                 140

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

Pro Val Val Leu Val Pro Gly Val Ile Ser Thr Gly Leu Glu Ser Trp
                165                 170                 175

Ser Leu Glu Gly Thr Glu Glu Cys Pro Thr Glu Ser His Phe Arg Lys
            180                 185                 190

Arg Met Trp Gly Ser Trp Tyr Met Ile Arg Val Met Leu Leu Asp Lys
        195                 200                 205

Tyr Cys Trp Leu Gln Asn Leu Met Leu Asp Thr Glu Thr Gly Leu Asp
    210                 215                 220

Pro Pro His Phe Lys Leu Arg Ala Ala Gln Gly Phe Ala Ser Ala Asp
225                 230                 235                 240

Phe Phe Met Ala Gly Tyr Trp Leu Trp Asn Lys Leu Leu Glu Asn Leu
                245                 250                 255

Ala Val Ile Gly Tyr Asp Thr Asp Thr Met Ser Ala Ala Ala Tyr Asp
            260                 265                 270

Trp Arg Leu Ser Tyr Pro Asp Leu Glu His Arg Asp Gly Tyr Phe Ser
        275                 280                 285

Lys Leu Lys Ala Ser Ile Glu Glu Thr Lys Arg Met Thr Gly Glu Lys
    290                 295                 300

Thr Val Leu Thr Gly His Ser Met Gly Ser Gln Val Ile Phe Tyr Phe
305                 310                 315                 320

Met Lys Trp Ala Glu Ala Glu Gly Tyr Gly Gly Gly Gly Pro Asn Trp
                325                 330                 335

Val Asn Asp His Ile Glu Ser Phe Val Asp Ile Ser Gly Ser Met Leu
            340                 345                 350

Gly Thr Pro Lys Thr Leu Val Ala Leu Leu Ser Gly Glu Met Lys Asp
        355                 360                 365

Thr Val Gln Leu Asn Ala Met Ala Val Tyr Gly Leu Glu Gln Phe Phe
    370                 375                 380

Ser Arg Arg Glu Arg Ala Asp Leu Leu Arg Thr Trp Gly Gly Ile Ala
385                 390                 395                 400

Ser Met Ile Pro Lys Gly Gly Lys Ala Ile Trp Gly Asp His Ser Gly
                405                 410                 415

Ala Pro Asp Asp Glu Pro Gly Gln Asn Val Thr Phe Gly Asn Phe Ile
            420                 425                 430

Lys Phe Lys Glu Ser Leu Thr Glu Tyr Ser Ala Lys Asn Leu Thr Met
        435                 440                 445

Asp Glu Thr Val Asp Phe Leu Tyr Ser Gln Ser Pro Glu Trp Phe Val
    450                 455                 460

Asn Arg Thr Glu Gly Ala Tyr Ser Phe Gly Ile Ala Lys Thr Arg Lys
465                 470                 475                 480

Gln Val Glu Gln Asn Glu Lys Arg Pro Ser Thr Trp Ser Asn Pro Leu
                485                 490                 495

Glu Ala Ala Leu Pro Asn Ala Pro Asp Leu Lys Ile Tyr Cys Phe Tyr
            500                 505                 510

Gly Val Gly Lys Asp Thr Glu Arg Ala Tyr Tyr Tyr Gln Asp Glu Pro
        515                 520                 525

Asn Pro Glu Gln Thr Asn Leu Asn Val Ser Ile Ala Gly Asn Asp Pro
    530                 535                 540

Asp Gly Val Leu Met Gly Gln Gly Asp Gly Thr Val Ser Leu Val Thr
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Asp Gly Val Leu Met Gly Gln Gly Asp Gly Thr Val Ser Leu Val Thr
545                 550                 555                 560

His Thr Met Cys His Arg Trp Lys Asp Glu Asn Ser Lys Phe Asn Pro

                565                 570                 575

Gly Asn Ala Gln Val Lys Val Val Glu Met Leu His Gln Pro Asp Arg
            580                 585                 590

Leu Asp Ile Arg Gly Gly Ala Gln Thr Ala Glu His Val Asp Ile Leu
        595                 600                 605

Gly Arg Ser Glu Leu Asn Glu Met Val Leu Lys Val Ala Ser Gly Lys
    610                 615                 620

Gly Asn Glu Ile Glu Glu Arg Val Ile Ser Asn Ile Asp Glu Trp Val
625                 630                 635                 640

Trp Lys Ile Asp Leu Gly Ser Asn
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<210> 33
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<213> Artificial Sequence

<220>
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<223> Xaa = Val [V] or Ala [A] or Asn [N] or Cys [C] or Gly [G] or Gln [Q] or His [H] or Leu [L] or Met [M] or Phe [F] or Pro [P] or Ser [S] or Thr [T] or Trp [W] or Tyr [Y] or Ile [I]

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<220>
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<223> Xaa = Lys [K] or Ala [A] or Arg [R] or Asn [N] or Gly [G] or His [H] or Pro [P] or Ser [S] or Thr [T] or Tyr [Y]

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<400> 33

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5
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<223> Xaa = Val [V] or Ala [A] or Asn [N] or Cys [C] or Gly [G] or Gln [Q] or His [H] or Leu [L] or Met [M] or Phe [F] or Pro [P] or Ser [S] or Thr [T] or Trp [W] or Tyr [Y] or Ile [I]

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<220>
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<223> Xaa = Leu [L] or Ala [A] or Asn [N] or Cys [C] or Gly [G] or Gln [Q] or His [H] or Ile [I] or Met [M] or Phe [F] or Pro [P] or Ser [S] or Thr [T] or Trp [W] or Tyr [Y] or Val [V]

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<223> Xaa can be any naturally occurring amino acid

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<400> 34
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Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                  10                  15
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Xaa

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<223> Xaa can be any naturally occurring amino acid

<220>
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Trp His Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10

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<220>
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<222> (7)..(8)
<223> Xaa can be any naturally occurring amino acid

<220>

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<223> Xaa = Pro [P] or Ala [A] or Arg [R] or Gly [G] or His [H] or Ile [I] or Leu [L] or Lys [K] or Met [M] or Phe [F] or Pro [P] or Ser [S] or Thr [T] or Trp [W] or Tyr [Y] or Val [V]

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<223> Xaa = Tyr [Y] or Ala [A] or Gly [G] or His [H] or Leu [L] or Phe [F] or Pro [P] or Ser [S] or Thr [T] or Val [V]

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<223> Xaa can be any naturally occurring amino acid

<220>
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<223> Xaa = Thr [T] or Phe [F] or Ala [A] or Cys [C] or Gly [G] or His [H[or Ile [I] or Leu [L] or Met [M] or Pro [P] or Ser [S] or Trp [W] or Tyr [Y] or Val [V]

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<400> 36

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Xaa Xaa Xaa Trp His Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                  10                  15
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<210> 37
<211> 512
<212> PRT
<213> Yarrowia lipolytica

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<223> Xaa = Val [V] or Cys [C]

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<223> Xaa = Leu [L] or Ala [A] or Cys [C] or Gly [G]

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<223> Xaa = Cys [C] or Asp [D] or Ile [I] or Phe [F]

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<223> Xaa = Met [M] or Gly [G] or Pro [P] or Ser [S] or Val [V] or Asn [N] or Thr [T]

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<223> Xaa = Leu [L] or Ala [A] or His [H] or Met [M] or Gly [G] or Ile [I] or Asn [N]

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<223> Xaa = Ser [S] or Leu [L] or Trp [W] or Gly [G] or Asn [N]

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<222> (140)..(140)
<223> Xaa = Ser [S] or Asn [N] or His [H] or Pro [P] or Trp [W] or Tyr [Y] or Ile [I]

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<223> Xaa = Phe [F] or Ala [A] or Met [M] or Trp [W] or Val [V]

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<222> (142)..(142)
<223> Xaa = Gly [G] or His [H] or Ile [I] or Val [V]

<220>
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<222> (143)..(143)
<223> Xaa = Trp [W] or Leu [L] or His [H]

<220>
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<222> (144)..(144)
<223> Xaa = Asn [N] or Ala [A] or Lys [K] or Phe [F] or Thr [T] or Val [V]

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<223> Xaa = Tyr [Y] or Gly [G] or Leu [L] or Met [M]

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<223> Xaa = Asp [D] or Asn [N] or Gln [Q] or His [H] or Glu [E]

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<223> Xaa = Gly [G] or Ala [A] or Asn [N] or Val [V]

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<223> Xaa = Phe [F] or Tyr [Y]

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<220>

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<223> Xaa = Arg [R] or Ala [A] or Met [M]

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<223> Xaa = Leu [L] or Gly [G] or Tyr [Y] or His [H] or Thr [T]

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<400> 37

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Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                  10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    130                 135                 140

Xaa Xaa Xaa Xaa Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
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    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Xaa Trp His Gly Xaa Xaa Pro
    370                 375                 380

Gly Tyr Tyr Xaa Xaa Xaa Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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<210> 38
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<220>
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<400> 38
Met Xaa Xaa Xaa Xaa Xaa Xaa
1 5

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<220>
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<223> Xaa can be any naturally occurring amino acid

<220>
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<223> Xaa = Asp [D] or Gln [Q] or Asn [N] or His [H]

<220>
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<222> (17)..(17)
<223> Xaa = Gly [G] or Ala [A] or Val [V] or Asn [N]

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Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa

<210> 40
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<220>
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<223> Xaa = Phe [F] or Asn [N] or Cys [C] or Gly [G] or Thr [T]

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Trp His Gly Xaa Xaa Xaa Gly Tyr Xaa Xaa Xaa Xaa
1 5 10

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Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
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Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
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<213> Yarrowia lipolytica

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<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], Glu [Q], His [H], Ile [I], Leu [L], Phe [F], Pro [P], Ser [S], Thr [T], Trp [W], Tyr [Y] or Val [V]

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Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
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Gly Ala Gln Xaa Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
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Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
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Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
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Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
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Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe Val Gln Val Asn Pro Lys Thr Glu Lys Tyr Lys Trp Asn Asn Val
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Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
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Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
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Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
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<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], Glu [Q], His [H], Leu [L], Met [M], Phe [F], Pro [P], Ser [S], Thr [T], Trp [W] or Tyr [Y]

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Xaa Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
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Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr

            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
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Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
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<213> Yarrowia lipolytica

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<222> (134)..(134)
<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], Glu [Q], His [H], Met [M], Phe [F], Pro [P], Ser [S], Thr [T], Trp [W], Tyr [Y] or Val [V]

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Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met

            20                  25                  30

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Xaa Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380
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Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
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<213> Yarrowia lipolytica

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<222> (135)..(135)

<223> Xaa = Arg [R], Asn [N], Asp [D], Gln [E], Gly [G], Glu [Q], His [H], Ile [I], Leu [L], Lys [K], Met [M], Phe [F], Pro [P], Ser [S], Trp [W] or Tyr [Y]

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1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Xaa Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
```

```
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 53

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (136)..(136)

<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], His [H], Ile [I], Phe [F], Pro [P], Ser [S], Thr [T], Trp [W], Tyr [Y] or Val [V]

<400> 53

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60
```

```
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Xaa Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480
```

```
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 54

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (137)..(137)

<223> Xaa = Ala [A], Arg [R], Asn [N], Gly [G], His [H], Pro [P], Ser [S], Thr [T] or Tyr [Y]

<400> 54

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Xaa Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320
```

```
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 55

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (138)..(138)

<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], Glu [Q], His [H], Ile [I], Met [M], Phe [F], Pro [P], Ser [S], Thr [T], Trp [W] or Tyr [Y]

<400> 55

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Xaa Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160
```

```
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg

            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 56

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (139)..(139)

<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], His [H], Leu [L], Met [M], Phe [F], Pro [P], Trp [W] or Val [V]

<400> 56

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Xaa Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu

        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400
```

```
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 57

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (140)..(140)

<223> Xaa = Asn [N], Cys [C], His [H], Ile [I], Leu [L], Phe [F], Pro [P], Trp [W], Tyr [Y] or Val [V]

<400> 57

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Xaa Phe Gly Trp Asn

    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220
```

```
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 58

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> misc

<222> (141)..(141)

<223> Xaa = Ala [A], Asn [N], Gly [G], His [H], Ile [I], Met [M], Pro [P], Ser [S], Thr [T], Trp [W] or Val [V]

<400> 58

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60
```

```
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser

65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Xaa Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480
```

```
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 59
<211> 512
<212> PRT
<213> Yarrowia lipolytica

<220>
<221> MISC_FEATURE
<222> (142)..(142)
<223> Xaa = Asn [N], His [H], Ile [I], Leu [L], Met [M], Phe [F], Pro [P], Thr [T], Trp [W], Tyr [Y] or Val [V]

<400> 59

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser

1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Xaa Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
```

```
Phe Ala Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 60

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (143)..(143)

<223> Xaa = Ala [A], Gly [G], His [H], Leu [L], Lys [K], Pro [P], Ser [S], Thr [T] or Val [V]

<400> 60

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Xaa Asn
    130                 135                 140

Val Trp Asp Glu Trp Gln Ile Glu Lys Glu Gly Gln Leu Ser Glu Phe
```

```
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 61

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (144)..(144)

<223> Xaa = Ala [A], Arg [R], Gly [G], His [H], Lys [K], Phe [F], Pro [P], Thr [T] or Val [V]

<400> 61

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                  10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Xaa
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415
```

```
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 62

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (145)..(145)

<223> Xaa = Ala [A], Cys [C], Gly [G], Gln [E], His [H], Met [M], Phe [F], Pro [P], Ser [S], Thr [T] or Trp [W]

<400> 62

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Xaa Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255
```

```
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 63

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> misc

<222> (146)..(146)

<223> Xaa = Arg [R], Asn [N], Asp [D], Gly [G], Gln [E], Glu [Q], Ile [I], Leu [L], Met [M], Phe [F], Pro [P], Trp [W]] or Val [V]

<400> 63

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95
```

```
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Xaa Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 64

<211> 512
<212> PRT
<213> Yarrowia lipolytica

<220>
<221> misc
<222> (147)..(147)
<223> Xaa = Ala [A], Asn [N], Gly [G], Gln [E], Glu [Q], His [H], Phe [F], Ser [S] or Thr [T]

<400> 64

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                  10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Xaa Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
```

```
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 65

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (148)..(148)

<223> Xaa = Ala [A], Asn [N], His [H], Leu [L], Met [M], Phe [F], Ser [S], Thr [T] or Val [V]

<400> 65

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Xaa Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205
```

```
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 66

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (376)..(376)

<223> Xaa = Ala [A], Gly [G], His [H], Leu [L], Phe [F], Pro [P], Thr [T] or Val [V]

<400> 66

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45
```

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
50 55 60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65 70 75 80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
85 90 95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
100 105 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
115 120 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
130 135 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145 150 155 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
165 170 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
195 200 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
210 215 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
245 250 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
260 265 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
275 280 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
290 295 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305 310 315 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
325 330 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
340 345 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
355 360 365

Thr Ile Phe Thr Phe Val Val Xaa Ala Phe Trp His Gly Thr Arg Pro
370 375 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385 390 395 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
405 410 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
420 425 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
435 440 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
450 455 460

```
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 67
<211> 512
<212> PRT
<213> Yarrowia lipolytica

<220>
<221> MISC_FEATURE
<222> (377)..(377)
<223> Xaa = Asn [N], Gly [G], His [H], Leu [L], Phe [F], Pro [P], Ser [S], Thr [T] or Val [V]

<400> 67

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300
```

```
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Xaa Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys

            500                 505                 510
```

<210> 68

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (378)..(378)

<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], His [H], Leu [L], Pro [P], Ser [S], Thr [T], Trp [W] or Tyr [Y]

<400> 68

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
```

```
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Xaa Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu

        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 69

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>
<221> MISC_FEATURE
<222> (382)..(382)
<223> Xaa = Ala [A], Asn [N], Gly [G], Glu [Q], His [H], Ile [I], Met [M], Pro [P], Ser [S], Trp [W] or Tyr [Y]

<400> 69

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                  10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Xaa Arg Pro
```

```
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 70

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (383)..(383)

<223> Xaa = Ala [A], Asn [N], Asp [D], Gly [G], Gln [E], Glu [Q], His [H], Ile [I], Leu [L], Lys [K], Met [M], Phe [F], Pro [P], Thr [T], Trp [W] or Val [V]

<400> 70

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val

    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asp Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
```

```
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Xaa Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 71

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (384)..(384)

<223> Xaa = Ala [A], Arg [R], Gly [G], His [H], Ile [I], Leu [L], Lys [K], Met [M], Phe [F], Ser [S], Thr [T], Trp [W], Tyr [Y] or Val [V]

<400> 71

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

```
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Xaa
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445
```

```
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 72

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (385)..(385)

<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], His [H], Ile [I], Leu [L], Lys [K], Met [M], Phe [F], Ser [S], Thr [T], Trp [W], Tyr [Y] or Val [V]

<400> 72

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285
```

```
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Xaa Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile

            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 73

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (386)..(386)

<223> Xaa = Ala [A], Gly [G], His [H], Leu [L], Phe [F], Pro [P], Ser [S], Thr [T] or Val [V]

<400> 73

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110
```

```
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala

        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Xaa Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 74

<211> 512

<212> PRT
<213> Yarrowia lipolytica

<220>
<221> MISC_FEATURE
<222> (387)..(387)
<223> Xaa = Ala [A], Gly [G], His [H], Leu [L], Phe [F], Pro [P], Ser [S], Thr [T], Trp [W] or Val [V]

<400> 74

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                  10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly

    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350
```

```
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Xaa Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 75

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (388)..(388)

<223> Xaa = Ala [A], Gly [G], His [H], Pro [P], Ser [S], Thr [T], Trp [W], Tyr [Y] or Val [V]

<400> 75

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190
```

```
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile

225                 230                 235                 240

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Xaa Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 76

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (389)..(389)

<223> Xaa = Ala [A], Cys [C], Gly [G], His [H], Ile [I], Leu [L], Met [M], Phe [F], Pro [P], Ser [S], Trp [W], Tyr [Y] or Val [V]

<400> 76

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15
```

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu

                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Xaa Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430
```

```
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 77

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<220>

<221> MISC_FEATURE

<222> (390)..(390)

<223> Xaa = Ala [A], Asn [N], Cys [C], Gly [G], His [H], Leu [L], Met [M], Pro [P], Ser [S], Thr [T] or Val [V]

<400> 77

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg

            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255
```

```
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Xaa Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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<210> 78

<211> 1539

<212> DNA

<213> Yarrowia lipolytica

<220>

<221> CDS

<222> (1)..(1539)

<400> 78

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct         48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg         96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa        144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg        192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca        240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg        288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

                                                                    336
```

```
gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt        336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc        384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt tct aag cta tcg tct ttt gga tgg aac        432
Gly Ala Gln Met Val Leu Cys Ser Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat gga tgg cag att gag aag ggt gag cag ctc agc gag ttc        480
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta        528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc        720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc        768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc        960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat       1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc       1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct       1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc gcg ttt gtg acc gct gcc atg tac cag tct gtt ggt       1200
Gly Tyr Tyr Leu Ala Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga       1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc       1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta       1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac       1392
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510

taa                                                                  1539
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<210> 79
<211> 512
<212> PRT
<213> Yarrowia lipolytica

<400> 79

```
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                  10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Ser Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380
```

```
Gly Tyr Tyr Leu Ala Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 80

<211> 1539

<212> DNA

<213> Yarrowia lipolytica

<220>

<221> CDS

<222> (1)..(1539)

<400> 80

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct         48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg         96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa        144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg        192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca        240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg        288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt        336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc        384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt tct aag cta tcg tct ttt gga tgg aac        432
Gly Ala Gln Met Val Leu Cys Ser Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat gga tgg cag att gag aag ggt gag cag ctc agc gag ttc        480
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta        528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu

        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220
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    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc        720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc        768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc        960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat       1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc       1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct       1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc tgc ttt gtg acc gct gcc atg tac cag tct gtt ggt       1200
Gly Tyr Tyr Leu Cys Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga       1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc       1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta       1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac       1392

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510

taa                                                                  1539
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<210> 81

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<400> 81

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Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80
```

```
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                  85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
             100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
         115                 120                 125

Gly Ala Gln Met Val Leu Cys Ser Lys Leu Ser Ser Phe Gly Trp Asn
     130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                 165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
         195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
     210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                 245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
             260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
         275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
     290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                 325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
             340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
         355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
     370                 375                 380

Gly Tyr Tyr Leu Cys Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly

                 405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
             420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
         435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
     450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
```

```
               485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 82

<211> 1539

<212> DNA

<213> Yarrowia lipolytica

<220>

<221> CDS

<222> (1)..(1539)

<400> 82

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct         48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg         96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa        144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg        192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca        240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg        288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt        336

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc        384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt tct aag cta tcg tct ttt gga tgg aac        432
Gly Ala Gln Met Val Leu Cys Ser Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat gga tgg cag att gag aag ggt gag cag ctc agc gag ttc        480
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta        528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc        720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc        768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc        960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
```

```
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat      1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc      1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct      1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc agc ttt gtg acc gct gcc atg tac cag tct gtt ggt      1200
Gly Tyr Tyr Leu Ser Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga      1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc      1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta      1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac      1392
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca      1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac      1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag      1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510

taa                                                                 1539
```

<210> 83

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<400> 83

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Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val

    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Ser Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175
```

```
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Ser Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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<210> 84

<211> 1539

<212> DNA

<213> Yarrowia lipolytica

<220>

<221> CDS

<222> (1)..(1539)

<400> 84

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct        48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15
```

```
aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg        96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa       144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg       192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca       240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg       288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt       336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc       384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt gtt aag cta tcg tct ttt gga tgg aac       432
Gly Ala Gln Met Val Leu Cys Val Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat gga tgg cag att gag aag ggt gag cag ctc agc gag ttc       480
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta       528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac       576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg       624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag       672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc       720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc       768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga       816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac       864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga       912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc       960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct      1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat      1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc      1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct      1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc tgc ttt gtg acc gct gcc atg tac cag tct gtt ggt      1200
Gly Tyr Tyr Leu Cys Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga      1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc      1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta      1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
```

```
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac       1392
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys

            500                 505                 510

taa                                                                  1539
```

<210> 85

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<400> 85

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Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Val Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
```

```
                290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Cys Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
```

<210> 86

<211> 1539

<212> DNA

<213> Yarrowia lipolytica

<220>

<221> CDS

<222> (1)..(1539)

<400> 86

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct        48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg        96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa       144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg       192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca       240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg       288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt       336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc       384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt atg aag cta tcg tct ttt gga tgg gct       432
Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Ala
```

```
    130                 135                 140

gtc tac gat gga tgg cag att gag aag ggt gag cag ctc agc gag ttc        480
Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe

145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta        528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc        720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc        768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc        960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat       1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc       1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct       1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc acc agc gtg acc gct gcc atg tac cag tct gtt ggt       1200
Gly Tyr Tyr Leu Thr Ser Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga       1248

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc       1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta       1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac       1392
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510

taa                                                                  1539
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<210> 87

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<400> 87

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Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Ala
    130                 135                 140

Val Tyr Asp Gly Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala

        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Ser Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400
```

```
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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<210> 88

<211> 1539

<212> DNA

<213> Yarrowia lipolytica

<220>

<221> CDS

<222> (1)..(1539)

<400> 88

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct         48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg         96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa        144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg        192
Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca        240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg        288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt        336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc        384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt atg aag cta tcg tct ttt gga tgg aac        432
Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat gct tgg cag att gag aag ggt gag cag ctc agc gag ttc        480
Val Tyr Asp Ala Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta        528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc        720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240
```

```
ttc tgg atc gtt ctg tgg acc cag gtg gac tct cga atc tcc acc gcc        768
Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc        960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat       1056
Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc       1104
Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct       1152
Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

ggc tac tat ctc acc agc gtg acc gct gcc atg tac cag tct gtt ggt       1200
Gly Tyr Tyr Leu Thr Ser Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga       1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc       1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta       1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac       1392
Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510

taa                                                                  1539
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<210> 89

<211> 512

<212> PRT

<213> Yarrowia lipolytica

<400> 89

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Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser

1               5                   10                  15

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

Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
    50                  55                  60

Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
65                  70                  75                  80
```

```
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
            100                 105                 110

Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

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

Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
                245                 250                 255

Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Ser Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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<210> 90
<211> 1539
<212> DNA
<213> Yarrowia lipolytica

<220>
<221> CDS
<222> (1)..(1539)

<400> 90

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atg gcc ttt cct tgg gca gat aag tgg gca gcc gat gcg tct gca tct         48
Met Ala Phe Pro Trp Ala Asp Lys Trp Ala Ala Asp Ala Ser Ala Ser
1               5                   10                  15

aca ggg ctg cct ccg gac ctc ctc aag att gca ttc act ctg gtc atg         96
Thr Gly Leu Pro Pro Asp Leu Leu Lys Ile Ala Phe Thr Leu Val Met
            20                  25                  30

tct tat ccg ctg agt tct ctc atg aaa cgg ctg cca gat gac gcc aaa        144
Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
        35                  40                  45

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Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
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ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca        240
Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
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atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg        288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
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gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt        336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
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tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc        384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
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gga gca cag atg gtt ctg tgt atg aag cta tcg tct ttt gga tgg aac        432
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gtc tac gat aat tgg cag att gag aag ggt gag cag ctc agc gag ttc        480
Val Tyr Asp Asn Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta        528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
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gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac        576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg        624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag        672
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tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga        816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
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att gtg tac ctc tac atg ctc ggt ttc atg tac cga ctc aag tac tac        864
Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
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gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga        912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
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Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct       1008
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Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr

    450                 455                 460

cac att gct ctg ggc gcc atc ttt gca att tct agc ccc tac aag gca       1440
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465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
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Ser Tyr Pro Leu Ser Ser Leu Met Lys Arg Leu Pro Asp Asp Ala Lys
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Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
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Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
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Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
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Val Tyr Asp Asn Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
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Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

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

Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
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Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
        275                 280                 285

Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

Leu Leu Glu Ala Trp Asn Gln Asn Thr Asn Lys Trp Leu Arg Asn Tyr
            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Ile Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Phe Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

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aac ctc aag atc atc tat atc atc tcc gtg tcc atc ttc tac atg gtg        192
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ggt gtc ttc tcc ctc tat ggc gga gct gcc act ctg ctc ttc tcc tca        240
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65                  70                  75                  80

atg ggt acc ttc ttc atc acc caa tgg aag agc cct tac atg ccc tgg        288
Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
                85                  90                  95

gtc aat ttt ggt ttt gtc atg acc cat ctc ttc gtc aat cac ctg cgt        336
Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg

           100                 105                 110

tcg cag ttt ttc ccc gaa aca tac gac ccc aat gtc att gac atc acc       384
Ser Gln Phe Phe Pro Glu Thr Tyr Asp Pro Asn Val Ile Asp Ile Thr
        115                 120                 125

gga gca cag atg gtt ctg tgt atg aag cta tcg tct ttt gga tgg aac       432
Gly Ala Gln Met Val Leu Cys Met Lys Leu Ser Ser Phe Gly Trp Asn
    130                 135                 140

gtc tac gat aat tgg cag att gag aag ggt gag cag ctc agc gag ttc       480
Val Tyr Asp Asn Trp Gln Ile Glu Lys Gly Glu Gln Leu Ser Glu Phe
145                 150                 155                 160

cag act aaa agg gct gtt ctc aag cac ccc agt ctt atg gac ttc cta       528
Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

gct ttt gtg ttc tac ttc cct tcc att ctg aca ggt cct tct tac gac       576
Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
            180                 185                 190

tat atg gag ttc cat aac tgg ctc gat ctc agc ctg ttc aag gag ctg       624
Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

gag aaa gat aag gac ccc aag cga gct gct cga cga aag cga cac aag       672
Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

atc ccc cga tct gga atc gct gct tcc aag aaa ctc gcc gct ggt atc       720
Ile Pro Arg Ser Gly Ile Ala Ala Ser Lys Lys Leu Ala Ala Gly Ile
225                 230                 235                 240

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tac gct tac tca gac gca ttc acc aag gag cac aac atc ttt gga cga       816
Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

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Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
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gga gcc tgg tcc att tcc gag gga gcc tgc atc ttg tct ggc ctc gga       912
Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
    290                 295                 300

ttc cac ggc gtg gac ccc aaa act ggc aag tac aag tgg gac cgt gtc       960
Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
305                 310                 315                 320

cag aac gtg gac ccg tgg gga ttc gaa act ggt caa aac aca aag gct      1008
Gln Asn Val Asp Pro Trp Gly Phe Glu Thr Gly Gln Asn Thr Lys Ala
                325                 330                 335

ctg ctg gag gcc tgg aac cag aac act aac aag tgg cta cga aac tat      1056
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gtg tac ctc cga gtg gtg ccc aaa ggc caa aag cct gga ttc cga gcc      1104

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
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act atc ttc aca ttt gtg gtt tcc gcc ttc tgg cat gga act cga cct      1152
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    370                 375                 380

ggc tac tat ctc acc agc gtg acc gct gcc atg tac cag tct gtt ggt      1200
Gly Tyr Tyr Leu Thr Ser Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

aag ttc ttc cga cga tac ctg cga ccc ttc ttc atg gag tct gat gga      1248
Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

aag act gcc ggt ccc tat aag atc tac tac gac att gtg tgt tgg atc      1296
Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
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gtt gtc caa acc gca ttt gga tac gct acc cag tcc ttt atg att cta      1344
Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

gac ttc tgg ctg tcg ctc aag tgt tgg aag aac tcc tgg ttc ctg tac      1392
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465                 470                 475                 480

tgg gcg att ccc aag atc aag aaa aag cag gct gga gcc gtc act gac       1488
Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

aag aag gac gcc aag gag gag gtg aag aag gac acc atc aag acc aag       1536
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Asn Leu Lys Ile Ile Tyr Ile Ile Ser Val Ser Ile Phe Tyr Met Val
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Gly Val Phe Ser Leu Tyr Gly Gly Ala Ala Thr Leu Leu Phe Ser Ser
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Met Gly Thr Phe Phe Ile Thr Gln Trp Lys Ser Pro Tyr Met Pro Trp
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Val Asn Phe Gly Phe Val Met Thr His Leu Phe Val Asn His Leu Arg
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Gln Thr Lys Arg Ala Val Leu Lys His Pro Ser Leu Met Asp Phe Leu
                165                 170                 175

Ala Phe Val Phe Tyr Phe Pro Ser Ile Leu Thr Gly Pro Ser Tyr Asp
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Tyr Met Glu Phe His Asn Trp Leu Asp Leu Ser Leu Phe Lys Glu Leu
        195                 200                 205

Glu Lys Asp Lys Asp Pro Lys Arg Ala Ala Arg Arg Lys Arg His Lys
    210                 215                 220

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

Phe Trp Ile Val Leu Trp Thr Gln Val Asp Ser Arg Ile Ser Thr Ala
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Tyr Ala Tyr Ser Asp Ala Phe Thr Lys Glu His Asn Ile Phe Gly Arg
            260                 265                 270

Ile Val Tyr Leu Tyr Met Leu Gly Phe Met Tyr Arg Leu Lys Tyr Tyr
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Gly Ala Trp Ser Ile Ser Glu Gly Ala Cys Ile Leu Ser Gly Leu Gly
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Phe His Gly Val Asp Pro Lys Thr Gly Lys Tyr Lys Trp Asp Arg Val
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            340                 345                 350

Val Tyr Leu Arg Val Val Pro Lys Gly Gln Lys Pro Gly Phe Arg Ala
        355                 360                 365

Thr Ile Phe Thr Phe Val Val Ser Ala Phe Trp His Gly Thr Arg Pro
    370                 375                 380

Gly Tyr Tyr Leu Thr Ser Val Thr Ala Ala Met Tyr Gln Ser Val Gly
385                 390                 395                 400

Lys Phe Phe Arg Arg Tyr Leu Arg Pro Phe Phe Met Glu Ser Asp Gly
                405                 410                 415

Lys Thr Ala Gly Pro Tyr Lys Ile Tyr Tyr Asp Ile Val Cys Trp Ile
            420                 425                 430

Val Val Gln Thr Ala Phe Gly Tyr Ala Thr Gln Ser Phe Met Ile Leu
        435                 440                 445

Asp Phe Trp Leu Ser Leu Lys Cys Trp Lys Asn Ser Trp Phe Leu Tyr
    450                 455                 460

His Ile Ala Leu Gly Ala Ile Phe Ala Ile Ser Ser Pro Tyr Lys Ala
465                 470                 475                 480

Trp Ala Ile Pro Lys Ile Lys Lys Lys Gln Ala Gly Ala Val Thr Asp
                485                 490                 495

Lys Lys Asp Ala Lys Glu Glu Val Lys Lys Asp Thr Ile Lys Thr Lys
            500                 505                 510
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FORBEDRET FREMSTILLING AF POLYUMÆTTEDE FEDTSYRER VED COEKSPRESSION AF ACYL-COA:LYSOPHOSPHATIDYLCHOLINACYLTRANSFERASER OG PHOSPHOLIPID:DIACYLGLYCEROLACYLTRANSFERASER

PATENTKRAV

1. Rekombinant *Yarrowia*-celle til fremstilling af mindst én langkædet, polyumættet fedtsyre med en kædelængde på C20 eller større, hvor den rekombinante celle er blevet genetisk manipuleret til at omfatte en biosyntetisk polyumættet bane, der er i stand til at fremstille mindst én langkædet, polyumættet fedtsyre og er yderligere modificeret til at indføre;

(a) et kimært gen, der koder for mindst ét polypeptid med acyl CoA:lysophosphatidylcholinacyltransferase- (LPCAT) aktivitet, hvor polypeptidet har mindst 90 % aminosyreidentitet, baseret på Clustal W-alignmentmetoden ved sammenligning med aminosyresekvensen ifølge SEQ ID NO: 4 (YILPCAT); og

(b) et kimært gen, der koder for mindst ét polypeptid med phospholipid:diacylglycerolacyltransferase- (PDAT) aktivitet, hvor polypeptidet har mindst 90 % aminosyreidentitet, baseret på Clustal W-alignmentmetoden, ved sammenligning med aminosyresekvensen ifølge SEQ ID NO: 32 (YIPDAT); og

hvor de kimære gener hvert omfatter en promoter, der er heterolog med den kodende sekvens, der koder for polypeptiderne fra (a) og (b), og hvor den rekombinante celle omfatter en øget mængde af den polyumættede fedtsyre målt som en vægtprocent af totale fedtsyrer ved sammenligning med en kontrolcelle, der svarer til den rekombinante *Yarrowia* celle til fremstilling, men som ikke er modificeret, til indføring af de kimære gener, der koder for LPCAT- eller PDAT-polypeptiderne fra (a) og (b).

2. Rekombinant *Yarrowia*-celle ifølge krav 1, hvor den rekombinant celle endvidere omfatter mindst én af følgende ved sammenligning med kontrolcellen:

(i) en øget C_{18}- til C_{20}-elongeringsomdannelseseffektivitet, eller

(ii) en øget mængde af totale fedtsyrer målt som en vægtprocent af tør cellevægt.

3. Rekombinant *Yarrowia*-celle ifølge krav 2, hvor den øgede C_{18}- til C_{20}-elongeringsomdannelseseffektivitet er en virkning af øget delta-9-elongaseomdannelseseffektivitet eller øget delta-6-elongaseomdannelseseffektivitet.

4. Rekombinant *Yarrowia*-celle ifølge krav 1, hvor polypeptidet, der har PDAT-aktivitet, har mindst 95 % aminosyreidentitet, baseret på Clustal W-alignmentmetoden ved sammenligning med en aminosyresekvens udvalgt fra gruppen bestående af SEQ ID NO: 32 (YIPDAT), og/eller hvor polypeptidet med acyl CoA:lysophosphatidylcholin-acyltransferaseaktivitet har mindst 95 % aminosyreidentitet, baseret på Clustal W-alignmentmetoden ved sammenligning med aminosyresekvensen ifølge SEQ ID NO: 4 (YILPCAT).

5. Rekombinant *Yarrowia*-celle ifølge krav 1, hvor polypeptidet med LPCAT-aktivitet er udvalgt fra gruppen bestående af:

a) et polypeptid, der omfatter mindst ét membranbundet mønster af O-acyltransferaseproteinfamilien, der er udvalgt fra gruppen bestående af: SEQ ID NO: 5 (WHG-X_3-GY-X_3-F), SEQ ID NO: 6 (Y-X_4-F), SEQ ID NO: 7 (Y-X_3-YF-X_2-H), SEQ ID NO: 8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-

X_8-DG), SEQ ID NO: 9 (RxKYY-X_2-W-X_3-[E/D]-[A/G]-X_5-GxG-[F/Y]-xG), SEQ ID NO: 10 (EX_{11}WN-X_2-[T/V]-X_2-W), SEQ ID NO: 11 (SAxWHG-X_2-PGY-X_2-[T/F]-F), SEQ ID NO: 12 (M-[V/I]-[L/I/V]-[V/C/A/T]-[M/L/Q]-K-[L/V/I/M]-[S/T/Y/I]-[S/T/A/M/G]-[F/L/C/Y]-[C/A/G/S]-[W/Y/M/I/F/C]-[N/S/E/Q/D]-[V/Y/L/I]-[H/Y/A/N/S/T]-DG), SEQ ID NO: 13 (R-[L/M/F/W/P/Y]-KYY-[G/A/F/H/S]-[V/A/I/C]-W-[Y/E/T/M/S/L]-[L/I/N]-[T/S/A]-[E/D]-[G/A]-[A/S/I/V]-[C/S/I/N/H/L]-[V/I/N]-[L/I/N/A/C]-[S/C/W/A/I]-G-[M/I/L/A/F]-G-[Y/F]-[N/E/S/T/R/K]-G), SEQ ID NO: 14 (E-[T/F/L/M]-[A/S]-[Q/D/P/K/T]-[N/S]-[S/I/T/L/A/M/F]-[H/K/R/V]-[G/C/E/T/Q/D/M]-[Y/A/M/L/I/F]-[L/S/P/I]-[G/E/A/L/N/D]-[S/A/V/F/M/N]-WN-[K/M/I/C]-[N/K/Q/G]-[T/V]-[N/A/S]-[H/K/N/T/R/L]-W), SEQ ID NO: 15 (SA-[F/M/V/I]-WHG-[F/V/T/L]

[Y/S/R]-PGY-[Y/M/I]-[L/M/I/F]-[T/F]-F), SEQ ID NO: 16 (M-[V/I]-L-X_2-KL), SEQ ID NO: 17 (RxKYY-X_2-W) og SEQ ID NO: 18 (SAxWHG); og

(b) et polypeptid, der omfatter mindst ét mutant membranbundet mønster af O-acyltransferaseproteinfamilien, der er udvalgt fra gruppen bestående af:

(i) et mutantmønster, der omfatter en aminosyresekvens ifølge SEQ ID NO: 38, hvor SEQ ID NO: 38 adskiller sig fra SEQ ID NO: 16 (M-[V/I]-L-X_2-KL) ved mindst én aminosyremutation udvalgt fra gruppen bestående af: V2C, I2C, L3A, L3C, L3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7I og L7M;

(ii) et mutantmønster, der omfatter en aminosyresekvens ifølge SEQ ID NO: 39, hvor SEQ ID NO: 39 adskiller sig fra SEQ ID NO: 8 (M-[V/I]-[L/I]-X_2-K-[L/V/I]-X_8-DG) ved mindst én aminosyremutation udvalgt fra gruppen bestående af: V2C, I2C, L3A, L3C, L3G, I3A, I3C, I3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7M, V7A, V7N, V7G, V7H, V7M, I7A, I7N, I7G, I7H, I7M, D16Q, D16N, D16H, G17A, G17V og G17N;

(iii) et mutantmønster, der omfatter en aminosyresekvens ifølge SEQ ID NO: 40, hvor SEQ ID NO: 40 adskiller sig fra SEQ ID NO: 5 (WHG-X_3-GY-X_3-F) med mindst én aminosyremutation udvalgt fra gruppen bestående af: F12N, F12C, F12G og F12T; og

(iv) et mutantmønster, der omfatter en aminosyresekvens ifølge SEQ ID NO: 41, hvor SEQ ID NO: 41 adskiller sig fra SEQ ID NO: 11 (SAxWHG-X_2-PGY-X_2-[T/F]-F) med mindst én aminosyremutation udvalgt fra gruppen bestående af: T14A, T14C, T14S, F14A, F14C, F14S, F15N, F15C, F15G og F15T.

6. Rekombinant *Yarrowia*-celle ifølge et hvilket som helst af kravene 1 til 5, hvor den langkædede, polyumættede fedtsyre er udvalgt fra gruppen bestående af: eicosadiensyre, dihomo-gamma-linolensyre, arachidonsyre, docosatetraensyre, omega-6-docosapentaensyre, eicosatriensyre, eicosatetraensyre, eicosapentaensyre, omega-3-docosapentaensyre og docosahexaensyre.

7. Fremgangsmåde til forbedring af fremstillingen af mindst én langkædet, polyumættet fedtsyre med en kædelængde på C_{20} eller større, hvilken fremgangsmåde omfatter:

(a) dyrkning af den rekombinante mikrobielle celle ifølge et hvilket som helst af kravene 1 til 6 i nærvær af en fermenterbar carbonkilde; og

(b) eventuel genvinding af den langkædede, polyumættede fedtsyre.

8. Fremgangsmåde ifølge krav 7, hvor den langkædede, polyumættede fedtsyre er udvalgt fra gruppen bestående af: eicosadiensyre, dihomo-gamma-linolensyre, arachidonsyre, docosatetraensyre, omega-6-docosapentaensyre, eicosatriensyre, eicosatetraensyre, eicosapentaensyre, omega-3-docosapentaensyre og docosahexaensyre.

DRAWINGS

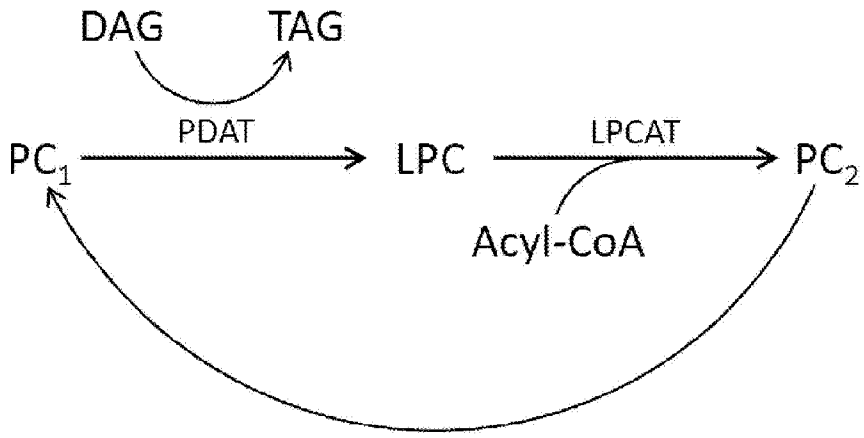


FIG. 1

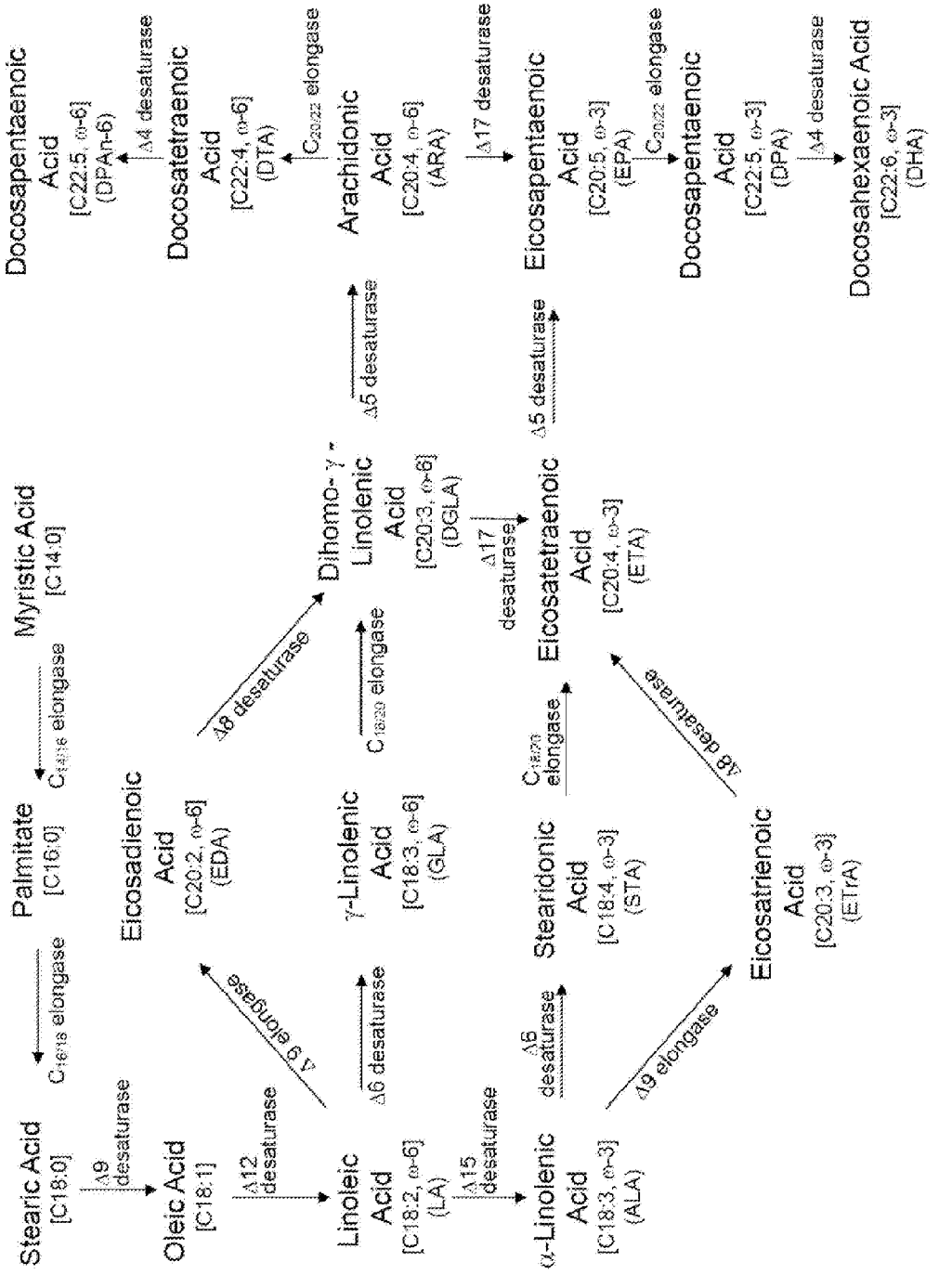
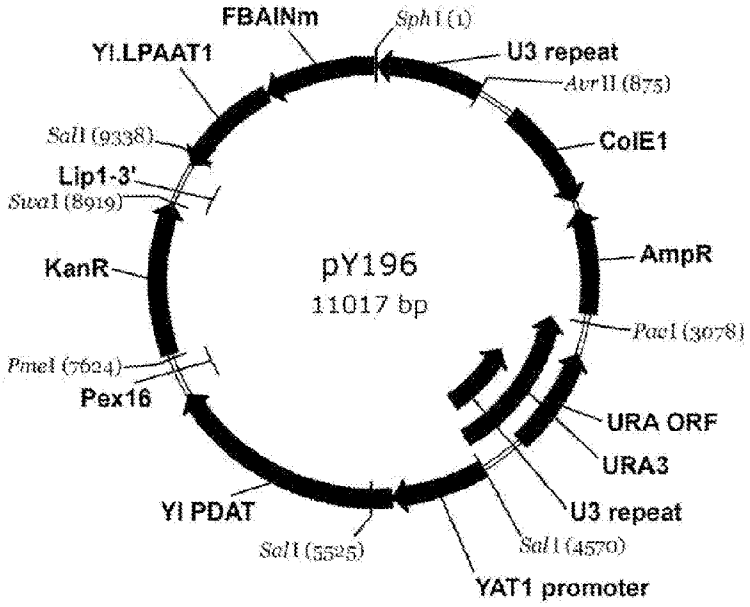

Docosapentaenoic Acid [C22:5, ω-6] (DPAn-6)
Δ4 desaturase
Docosatetraenoic Acid [C22:4, ω-6] (DTA)
C20/22 elongase
Arachidonic Acid [C20:4, ω-6] (ARA)
Δ17 desaturase
Eicosapentaenoic Acid [C20:5, ω-3] (EPA)
C20/22 elongase
Docosapentaenoic Acid [C22:5, ω-3] (DPA)
Δ4 desaturase
Docosahexaenoic Acid [C22:6, ω-3] (DHA)
Δ5 desaturase
Δ5 desaturase
Eicosatetraenoic Acid [C20:4, ω-3] (ETA)
Δ17 desaturase
Dihomo-γ-Linolenic Acid [C20:3, ω-6] (DGLA)
Δ8 desaturase
C18/20 elongase
γ-Linolenic Acid [C18:3, ω-6] (GLA)
Δ6 desaturase
Eicosadienoic Acid [C20:2, ω-6] (EDA)
Δ6 elongase
Palmitate [C16:0]
C16/18 elongase
Myristic Acid [C14:0]
C14/16 elongase
Stearic Acid [C18:0]
Δ9 desaturase
Oleic Acid [C18:1]
Δ12 desaturase
Linoleic Acid [C18:2, ω-6] (LA)
Δ15 desaturase
α-Linolenic Acid [C18:3, ω-3] (ALA)
Δ6 desaturase
Stearidonic Acid [C18:4, ω-3] (STA)
C18/20 elongase
Δ9 elongase
Eicosatrienoic Acid [C20:3, ω-3] (ETrA)
Δ8 desaturase

FIG. 2

A)


FBAINm
SphI (1)
Yl.LPAAT1
U3 repeat
AvrII (875)
SalI (9338)
ColE1
Lip1-3'
SwaI (8919)
pY196
11017 bp
AmpR
KanR
PacI (3078)
PmeI (7624)
URA ORF
Pex16
URA3
U3 repeat
Yl PDAT
SalI (5525)
SalI (4570)
YAT1 promoter

B)

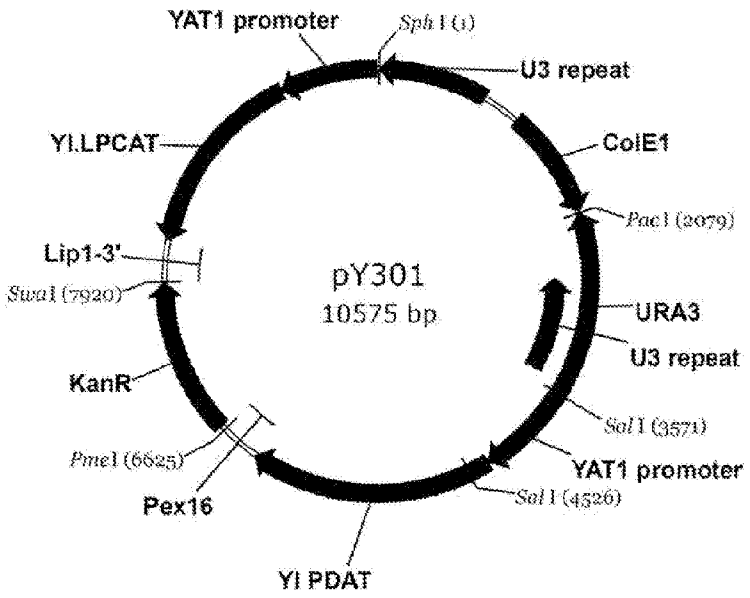

YAT1 promoter
SphI (1)
U3 repeat
Yl.LPCAT
ColE1
PacI (2079)
Lip1-3'
pY301
10575 bp
SwaI (7920)
URA3
U3 repeat
KanR
SalI (3571)
PmeI (6825)
YAT1 promoter
SalI (4526)
Pex16
Yl PDAT

FIG. 3

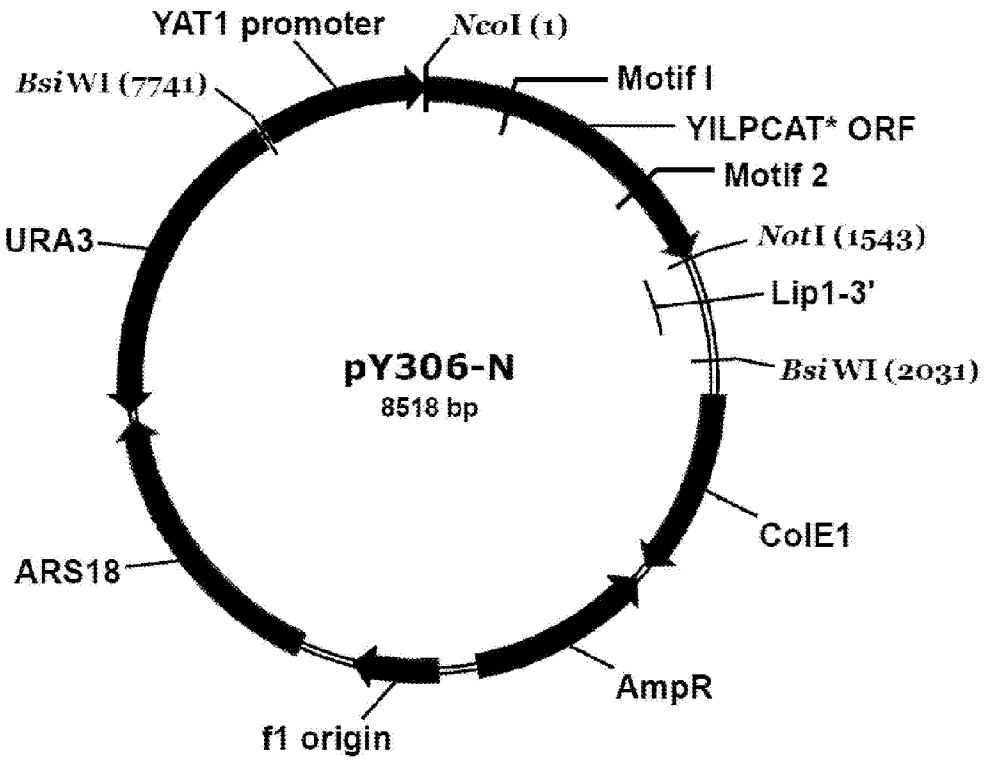

YAT1 promoter
NcoI (1)
BsiWI (7741)
Motif I
YILPCAT* ORF
Motif 2
URA3
NotI (1543)
Lip1-3'
pY306-N
8518 bp
BsiWI (2031)
ColE1
ARS18
AmpR
f1 origin

FIG. 4