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(71) Applicant: **E. I. DU PONT DE NEMOURS AND COMPANY** [US/US]; 1007 Market Street, Wilmington, Delaware 19898 (US).

(72) Inventors: **BOSTICK, Michael, W.**; 2650 Bittersweet Drive, Wilmington, Delaware 19810 (US). **YADAV, Narendra, S.**; 126 Tuckaway Drive, Wilmington, Delaware 19803 (US). **ZHANG, Hongxiang**; 3808 Rotherfield Lane, Chadds Ford, Pennsylvania 19317 (US). **ZHU, Quinn, Qun**; 544 Revere Road, West Chester, Pennsylvania 19382 (US).

(74) Agent: **CHRISTENBURY, Lynne, M.**; E. I. du Pont de Nemours and Company, Legal Patent Records Center, 4417 Lancaster Pike, Wilmington, Delaware 19805 (US).

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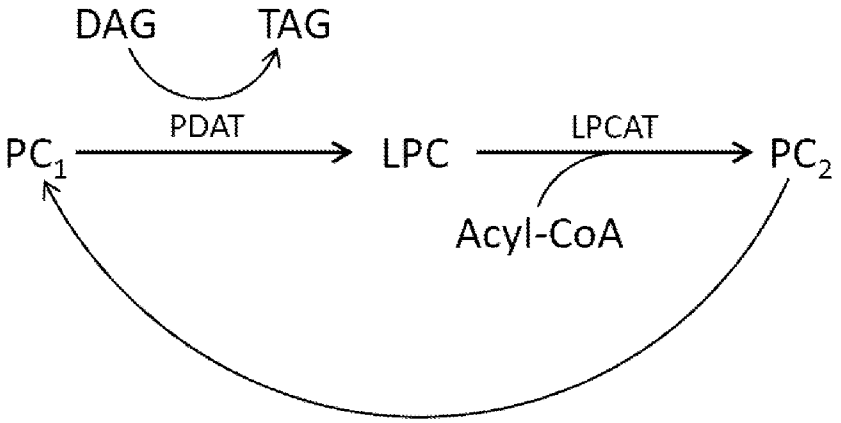

FIG. 1

(57) Abstract: Mutant acyl-CoA:lysophosphatidylcholine acyltransferases ["LPCATs"] having the ability to convert acyl-CoA + 1-acyl-*sn*-glycero-3-phosphocholine to CoA + 1,2-diacyl-*sn*-glycero-3-phosphocholine (EC 2.3.1.23) are disclosed herein. Isolated nucleic acid fragments and recombinant constructs comprising such fragments encoding mutant LPCATs, along with a method of making long chain polyunsaturated fatty acids ["PUFAs"] using these mutant LPCATs in oleaginous yeast, are also disclosed.

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TITLE

MUTANT ACYL-CoA:LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASES

This application claims the benefit of U.S. Provisional Application Nos. 61/661,615 and 61/661,623, each filed June 19, 2012, both of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

This invention is in the field of biotechnology. More specifically, this invention pertains to the creation of polynucleotide sequences encoding variant or mutant acyl-CoA:lysophosphatidylcholine acyltransferases and the use of these acyltransferases in making long-chain polyunsaturated fatty acids ["PUFAs"].

BACKGROUND OF THE INVENTION

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.

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].

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))

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).

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.

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.

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

In one embodiment, the invention concerns an isolated polynucleotide encoding a non-naturally occurring mutant polypeptide having acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) activity. 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.

In a second embodiment, the mutant membrane-bound *O*-acyltransferase protein motif is selected from the group consisting of:

(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 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;

(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 one amino acid 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, 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;

(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 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; and

(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 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 and 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.

A third embodiment concerns a mutant polypeptide encoded by the isolated polynucleotide of the invention, wherein the polypeptide has LPCAT activity.

A fourth embodiment concerns a recombinant cell comprising the isolated polynucleotide of the invention and a polyunsaturated fatty acid (PUFA) biosynthetic pathway capable of producing at least one long-chain PUFA, wherein the isolated polynucleotide is over-expressed in the recombinant cell. The recombinant cell also comprises at least one of the following:

(a) an amount of at least one long-chain PUFA measured as a weight percent of total fatty acids that is at least the same as the amount of the long-chain PUFA measured as a weight percent of total fatty acids in a control cell, or

(b) a C_{18} to C_{20} elongation conversion efficiency that is at least the same as the C_{18} to C_{20} elongation conversion efficiency of a control cell.

A fifth embodiment concerns a recombinant construct comprising the isolated polynucleotide of the invention.

A sixth embodiment concerns a transformed cell comprising a recombinant construct that comprises the isolated polynucleotide of the invention. Preferably, the transformed cell is selected from the group consisting of microbes and plants. More preferably, the transformed cell is an oleaginous yeast. Preferably, the oleaginous yeast is of the genus *Yarrowia*.

In a seventh embodiment, the mutant polypeptide encoded by the polynucleotide of the invention comprises an amino acid sequence as set forth in SEQ ID NO:19, wherein SEQ ID NO:19 differs from SEQ ID NO:4 (YlLPCAT) by at least one amino acid mutation, and wherein:

(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;

(ii) the amino acid mutation is 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

(iii) there are at least two amino acid mutations, wherein:

(1) a first amino acid mutation is an amino acid substitution selected from the group set forth in part (i), and

(2) a second amino acid mutation is an amino acid substitution selected from the group set forth in part (ii).

Further concerning this embodiment, overexpression of the mutant polypeptide in a recombinant *Yarrowia* cell comprising a PUFA biosynthetic pathway capable of producing at least one long-chain PUFA produces a result selected from the group consisting of:

(i) an amount of at least one long-chain PUFA measured as a weight percent of total fatty acids that is at least the same as the amount of the long-chain PUFA measured as a weight percent of total fatty acids in a control *Yarrowia* cell, and

(ii) a C_{18} to C_{20} elongation conversion efficiency that is at least the same as the C_{18} to C_{20} elongation conversion efficiency of a control *Yarrowia* cell.

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 an eighth embodiment, the mutant polypeptide encoded by the polynucleotide of the invention comprises 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 the difference is selected from the group consisting of:

(a) 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 and F390C;

(b) an amino acid mutation selected from the group consisting of: V133C, M136N, L138G, L138I, L138N, S139G, S139N, W143H, G148V, L388H, L388T, F390G, F390N and F390T;

(c) an amino acid mutation selected from the group consisting of: C135F, M136T, S140Y, S140I, F141V, G142I, G142V, D147E, F378Y, T382Y, R383A and F390S;

(d) a pair of amino acid mutations selected from the group consisting of:

(i) L134A and a second mutation selected from the group consisting of: L388G and F390T;

(ii) M136S and a second mutation selected from the group consisting of: F378Y, T382I, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C and T389S;

(iii) M136V and a second mutation selected from the group consisting of: T382P, T382Y, P384A, L388Y, T389A, T389C and T389S;

(iv) K137H and a second mutation selected from the group consisting of: P384G, L388T, F390S and F390T;

(v) K137N and a second mutation selected from the group consisting of: T382P, R383M, P384G, F378Y, L388G, L388T, T389A, T389S, F390S and F390T;

(vi) S140H and a second mutation selected from the group consisting of: P384G, L388T, F390G and F390S;

(vii) S140W and a second mutation selected from the group consisting of: T382I, T382P, T382Y, R383M, P384A, L388Y, T389A and T389C;

(viii) F141M and a second mutation selected from the group consisting of: F378Y, T382Y, R383M, P384A and T389C;

(ix) F141W and a second mutation selected from the group consisting of: F378Y, T382P, T382Y, R383M, P384A, T389A, T389C and T389S;

(x) N144A and a second mutation selected from the group consisting of: P384G, L388G, L388T, F390G, F390S and F390T;

(xi) N144T and a second mutation selected from the group consisting of: F378Y, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C and T389S;

(xii) V145M and a second mutation selected from the group consisting of: T382I, R383M, T389A and T389C;

(xiii) V145W and T382I;

(xiv) D147H and a second mutation selected from the group consisting of: L388G, L388T and F390S;

(xv) D147Q and a second mutation selected from the group consisting of: T382I and F390S;

(xvi) G148A and a second mutation selected from the group consisting of: F378Y, T382I, T382Y, R383M, P384G, L388G, L388Y, T389A, T389C, F390S and F390T; and

(xvii) G148N and a second mutation selected from the group consisting of: T382I, L388T, F390G and F390S;

(e) a pair of amino acid mutations selected from the group consisting of:

(i) L134A and F390G;

(ii) L134G and a second mutation selected from the group consisting of: F390G, L388G and F390T;

(iii) K137H and a second mutation selected from the group consisting of: T382I and F390G;

(iv) S140W and T389S;

(v) F141M and T389A;

(vi) N144A and T382I;

(vii) V145W and a second mutation selected from the group consisting of: T389S and T389A;

(viii) D147Q and a second mutation selected from the group consisting of: L388T and L388G;

(ix) D147H and F390T; and

(x) G148N and P384G; and

(f) a pair of amino acid mutations selected from the group consisting of:

(i) L134A and T382I;

(ii) K137H and L388G;

(iii) K137N and a second mutation selected from the group consisting of: T389C and F390G;

(iv) S140H and a second mutation selected from the group consisting of: T382I and L388G;

(v) F141W and a second mutation selected from the group consisting of: T382I and L388Y;

(vi) F141M and T382P;

(vii) V145M and a second mutation selected from the group consisting of: T382Y and F378Y;

(viii) V145W and F378Y;

(ix) D147 and T382I;

(x) G148A and P384A;

(xi) M136S and T389A; and

(xii) G148N_F390S.

In a ninth embodiment, the mutant polypeptide encoded by the polynucleotide of the invention comprises a sequence selected from the group consisting of: SEQ ID NOs:79, 81, 83, 85, 87, 89, 91 and 93.

A tenth embodiment concerns a transformed *Yarrowia* cell comprising the isolated polynucleotide of the invention, wherein oil produced by the transformed *Yarrowia* cell comprises at least one long-chain PUFA 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.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

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.

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	--	26

EGTR		
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 YILPCAT, 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 YILPCAT, comprising a single mutation in Motif I and a single mutation in Motif II	--	42 (512 AA)
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	--	51

Motif I		
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, S140I, S140L, S140F, S140P, S140W, S140Y or S140V mutation in Motif I	--	57
YILPCAT_F141X, comprising F141A, F141N, F141G, F141H, F141I, F141M, F141P, F141S, F141T, F141W, or F141V mutation in Motif I	--	58
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 and S376V	--	66

mutations		
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, P384M, P384F, P384S, P384T, P384W, P384Y or P384V mutation in Motif II	--	71
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 Y386V mutation in Motif II	--	73
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

All patents, patent applications and publications cited herein are incorporated herein by reference in their entirety.

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.

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.

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), which are herein incorporated by reference.

“Open reading frame” is abbreviated as “ORF”.

“Polymerase chain reaction” is abbreviated as “PCR”.

“American Type Culture Collection” is abbreviated as “ATCC”.

“Polyunsaturated fatty acid(s)” is abbreviated as “PUFA(s)”.

“Long-chain polyunsaturated fatty acid(s)” is abbreviated as “LC-PUFA(s)”.

“Triacylglycerols” are abbreviated as “TAGs”.

"Total fatty acids" are abbreviated as "TFAs".

"Fatty acid methyl esters" are abbreviated as "FAMEs".

"Dry cell weight" is abbreviated as "DCW".

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

"Lysophosphatidylcholine acyltransferase(s)" is abbreviated as "LPCAT(s)".

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

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

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"].

"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"].

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

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)).

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.).

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.

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.

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.

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.

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

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.

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]).

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.

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 [YlLPCAT] by at least one amino acid mutation, wherein:

(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;

(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

(c) said at least one amino acid mutation comprises at least two amino acid mutations, wherein:

(i) the first amino acid mutation is in an amino acid residue selected from the group set forth in part (a), and

(ii) the second amino acid mutation is in an amino acid residue selected from the group set forth in part (b).

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.

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).

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 (YlLPAAT1) 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 YlLPAAT1.

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

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.

The terms “YlLPAAT1” and “YlLPAAT2” refer to LPAATs isolated from *Yarrowia lipolytica.* An YlLPAAT 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 YlLPAAT1, but the other LPAAT motifs are not.

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)).

The term “YlPDAT” refers to a PDAT isolated from *Yarrowia lipolytica.* YlPDAT 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 which is incorporated herein by reference).

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.

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.

"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.

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.

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.

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.

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).

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.

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.

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, which is incorporated herein by reference.

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
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
Eicosa-tetraenoic	ETA	*cis*-8, 11, 14, 17-eicosatetraenoic	20:4 omega-3
Eicosa-pentaenoic	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
Docosa-pentaenoic	DPAn-6	*cis*-4, 7, 10, 13, 16-docosapentaenoic	22:5 omega-6
Docosa-pentaenoic	DPA	*cis*-7, 10, 13, 16, 19-docosapentaenoic	22:5 omega-3
Docosa-hexaenoic	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.

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.

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 which is incorporated herein by reference). 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.

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.

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.

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.

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(%)".

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.).

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.

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.

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.

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.

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

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.

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.

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.

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), which is incorporated herein by reference, particularly Chapter 11 and Table 11.1.

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.

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.

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.

“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

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.

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).

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.

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.

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.

“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.

“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 which is incorporated herein by reference.

"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.

"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.

"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.

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.

"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.

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.

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.

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).

"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.

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.

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.

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.

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.

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).

In a first embodiment, described herein is a recombinant microbial cell for the production of at least one long-chain (LC) polyunsaturated fatty acid (PUFA), said recombinant microbial cell comprising:

(a) at least one polypeptide having LPCAT activity;

(b) at least one polypeptide having PDAT activity; and

(c) a PUFA biosynthetic pathway capable of producing at least one long-chain polyunsaturated fatty acid;

wherein the polypeptides of (a) and (b) are over-expressed, and wherein the recombinant microbial cell has an increased amount ofat least one long-chain polyunsaturated fatty acid measured as a weight percent of total fatty acids ["wt. % TFAs"], when compared to a control cell.

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.

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.

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).

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.

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.

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.

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.

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.

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.

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:30 and 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 selected from the group consisting of SEQ ID NO:30 and 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 *Saccharomyces cerevisiae* or *Yarrowia lipolytica.*

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.

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.

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.

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.

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 is preferably selected from the group consisting of:

(a) a polypeptide having at least 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 (ScLPCAT) and SEQ ID NO:4 (YlLPCAT);

(b) 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-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), and SEQ ID NO:18 (SAxWHG);

(c) a polypeptide comprising at least one mutant membrane bound *O*-acyltransferase protein family motif, as described below;

(d) a polypeptide having at least 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 (YlLPAAT1) and SEQ ID NO:24 (ScLPAAT); and

(e) a polypeptide comprising at least one 1-acyl-*sn*-glycerol-3-phosphate acyltransferase family motif selected from the group

consisting of: SEQ ID NO:25 (NHxxxxD) and SEQ ID NO:26 (EGTR).

Alternatively, the polypeptide having LPCAT activity may have at least 90%, or 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:2 (ScLPCAT) and SEQ ID NO:4 (YlLPCAT). In this sense, 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.*

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.

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.

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).

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).

In one aspect of the present invention, 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:

(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;

(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, 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, 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;

(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;

(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

(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.

Therefore, one aspect of the invention concerns 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.

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.

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.

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.

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-X_2-PGY-X_2-[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.

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.

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-X_2-PGY-X_2-[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 YlLPCAT has been reported to be a likely active site residue (Lee et al., 2008, *Mol. Biol. Cell* 19:1174-1184).

Thus, in one embodiment herein, disclosed 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:

(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 (YlLPCAT) by at least one amino acid mutation, wherein:

(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;

(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

(iii) there are at least two amino acid mutations, wherein:

(1) a first amino acid mutation is an amino acid substitution selected from the group set forth in part (i), and

(2) the second amino acid mutation is an amino acid substitution selected from the group set forth in part (ii);

(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:

(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

(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.

Mutant polypeptides having LPCAT activity encoded by the isolated polynucleotide described above are also envisioned by the Applicants herein.

In one preferred embodiment, the amino acid sequence of a mutant YlLPCAT polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:37, wherein SEQ ID NO:37 differs from SEQ ID NO:4 (YlLPCAT) 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.

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:

(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, I2C, L3A, L3C, L3G, K6H, K6G, K6N, K6Y, L7A, L7N, L7G, L7H, L7I and L7M;

(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;

(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

(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.

The specific mutations set forth above correspond to mutations identified within YlLPCAT 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.

Following the work set forth above, wherein single amino acid mutations were created within either Motif I or Motif II of YlLPCAT (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 YlLPCAT.

Again, the effect of each double mutation on the LPCAT activity of the resulting mutant YlLPCAT protein was screened, based on EPA % TFAs and % delta-9 conversion efficiency. Most of the 167 YlLPCAT mutants functioned with approximately equal or improved activity when compared to YlLPCAT. 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.

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 YlLPCAT comprising a single mutation within Motif I and a single mutation within Motif II.

Thus, disclosed herein is the amino acid sequence of a mutant YlLPCAT 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 (YlLPCAT) 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. YlLPCAT 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 of 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).

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 YlLPCAT 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 YlLPCAT mutants found to exhibit equivalent or improved EPA % TFAs and equivalent or improved % Conv.

In one aspect of the invention, a mutant LPCAT polypeptide encoded by the isolated polynucleotide comprises a sequence selected from the group consisting of: SEQ ID NOs:79, 81, 83, 85, 87, 89, 91 and 93.

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.

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.

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:

(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

(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.

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.

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.

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.

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.

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).

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.

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.

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.

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.

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.

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.

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)), which are incorporated herein by reference. 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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).

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)).

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, all of which are incorporated herein by reference. 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.

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.

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.

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)).

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.

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)).

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.

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.

Thus, in one aspect, the present invention is directed toward a method for improving the production of at least one LC polyunsaturated fatty acid, comprising:

(a) growing the recombinant microbial cell of the invention in the presence of a fermentable carbon source; and

(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

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

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).

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.

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).

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, which is incorporated herein by reference.

Transformation of *Y. lipolytica* was performed as described in U.S. Pat. Appl. Publ. No. 2009-0093543-A1, which is incorporated herein by reference.

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.

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

Alternately, a modification of the base-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).

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.

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.

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.

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).

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

U.S. Pat. Appl. Publ. No. 2010-0317882-A1, incorporated herein by reference, describes the identification of a *Y. lipolytica* homolog to the *Saccharomyces cerevisiae* Ale1 (i.e., "ScAleI"; 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 YlAleI or YlLPCAT (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 ScAleI.

YlLPCAT 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, which are herein incorporated by reference. Specifically, these motifs are SEQ ID NOs:8-15 (Table 2). The His residue in SEQ ID NO:11 (SAxWHG-X_2-PGY-X_2-[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 YlLPCAT comprises at least the motifs SEQ ID NOs:8-11. It is hypothesized herein that these conserved motifs are likely involved in catalysis.

Overexpression of YlLPLAT 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*

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

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.

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::YlLPAAT1::Lip1 gene for expressing wild type *Y. lipolytica* LPAAT1 (SEQ ID NO:23), while pY301 also contained a chimeric YAT1::YlLPCAT::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 1-875	Fragment of *Y. lipolytica URA3* gene (GenBank Accession No. AJ306421; labeled as "U3 repeat" in Figure 3A)

*Avr*II/*Pac*I 875-3078	• ColE1 plasmid origin of replication • Ampicillin-resistance gene
*Pac*I/*Sal*I 3078-4570	*Y. lipolytica URA3* gene (GenBank Accession No. AJ306421)
*Sal*I/*Pme*I 4570-7624	YAT1::YIPDAT::PEX16, comprising: • YAT1: *Y. lipolytica* YAT1 promoter (U.S. Pat. Appl. Publ. No. 2010/0068789); • YIPDAT: *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. Y14439)
*Swa*I/*Sph*I 8919-1	FBAINm::YILPAAT1::Lip1 (complementary), comprising: • 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 1-875	Fragment of *Y. lipolytica URA3* gene (GenBank Accession No. AJ306421; labeled as “U3 repeat” in Figure)
*Avr*II/*Pac*I 875-2079	ColE1 plasmid origin of replication
*Pac*I/*Sal*I 2079-3571	*Y. lipolytica URA3* gene (GenBank Accession No. AJ306421)
*Sal*I/*Pme*I 3571-6625	YAT1::YIPDAT::PEX16 (as described in Table 5 for pY196)
*Pme*I/*Swa*I 6625-7920	Kanamycin-resistance gene from plasmid pBHR1 (GenBank Accession No. Y14439)
*Swa*I/*Sph*I 7920-1	YAT1::YILPCAT::Lip1 (complementary), comprising: • 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. lipolytica* Strain Z5567U19 Transformed with pY196 or pY301

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, which is incorporated herein by reference. A control transformation was also performed in which no plasmid DNA was included.

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.

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
	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

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.

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.

5 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

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

Previous attempts to enhance lipids in *Yarrowia* by other strategies
10 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
15 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.

20 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

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.

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).

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

YILPCAT lacking its internal *Nco*I sites (i.e., YILPCAT*) 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 YILPCAT).

YILPCAT* 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 1-2809	YAT1::YILPCAT*::Lip1 (complementary), comprising: • YAT1: *Y. lipolytica* YAT1 promoter (U.S. Pat. Appl. Publ. No. 2010/0068789); • YILPCAT*: 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/*EcoR*I 2809-5605	• ColE1 plasmid origin of replication • Ampicillin-resistance gene • f1 origin of replication
*EcoR*I/*Pac*I 5605-7021	*Y. lipolytica URA3* gene (GenBank Accession No. AJ306421)

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

EXAMPLE 4

Designing and Synthesizing Mutant YILPCAT Enzymes with Modified Motifs

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.

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 YlLPCAT 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

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 YlLPCAT 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 YlLPCAT 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, T382W, T382Y	69
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

Each of the 329 YlLPCAT 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 YlLPCAT Having Improved LPCAT Activity

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

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 YlLPCAT 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 YlLPCAT protein.

Transformation of *Y. lipolytica* Strain Y8406U2

Strain Y8406U2 was transformed to individually express one of each of the pY306-N vectors containing a mutant YlLPCAT 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, which is incorporated herein by reference. 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 YlLPCAT

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 YlLPCAT (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 YlLPCAT protein expression ["WT"]).

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.

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).

Comparison of each mutant's performance relative to the wild type YlLPCAT 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 Elongase Conversion Efficiency in Batch #1 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

Mutant	#	% TFAs												EPA SD	% Conv.
		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**
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 Elongase Conversion Efficiency in Batch #2 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

Mutant	#	% TFAs												EPA SD	% Conv.
		16:0	16:1	18:0	18:1	LA	ALA	EDA	DGLA	ARA	ETrA	ETA	EPA		
WT	5	3.0	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**
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 Elongase Conversion Efficiency in Batch #3 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

		% TFAs												EPA	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
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**
G148A +	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**
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	%
Mutant	**#**	**16:0**	**16:1**	**18:0**	**18:1**	**LA**	**ALA**	**EDA**	**DGLA**	**ARA**	**ETrA**	**ETA**	**EPA**	**SD**	**Conv.**
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**
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**
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 Elongase Conversion Efficiency in Batch #5 Transformants Comprising a Vector Encoding YILPCAT Having a Single Amino Acid Substitution

Mutant	#	% TFAs												EPA SD	% Conv.
		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**
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**
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

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 YlLPCAT). Also, all of the mutant YlLPCAT transformants had a % Conv. that was at least 87.6% of the % Conv. measured in the control.

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.

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 YILPCAT transformants relative to the EPA % TFAs and % Conv. in the wild type YILPCAT control transformants. An additional 8 YILPCAT 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

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

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

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.

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 Elongase Conversion Efficiency in Batch #6 Transformants Comprising a Vector Encoding YILPCAT Having Double Amino Acid Substitutions

Mutant	#	% TFAs												EPA SD	% Conv.
		16:0	16:1	18:0	18:1	LA	ALA	EDA	DGLA	ARA	ETrA	ETA	EPA		
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
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

Mutant	#	% TFAs												EPA SD	% Conv.
		16:0	16:1	18:0	18:1	LA	ALA	EDA	DGLA	ARA	ETrA	ETA	EPA		
WT	12	3.2	0.7	2.6	4.2	14.2	0.9	2.3	3.0	0.7	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**
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 Elongase 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**
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 Elongase 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
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

Based on the data set forth above, it is clear that most of the 167 YlLPCAT 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.

More specifically, 106 YlLPCAT 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).

An additional 15 YlLPCAT double mutants (of the 167 analyzed) had equivalent or improved EPA % TFAs when compared to the control, while an additional 6 YlLPCAT 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

A total of 23 YlLPCAT 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.

Transformants expressing these double mutant YlLPCAT 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.

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.

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 Elongase 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	2.9	**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 Elongase Conversion Efficiency in Selected Transformants Comprising a Vector Encoding YlLPCAT 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

Of the 25 YlLPCAT 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.

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

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.

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

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.

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.

CLAIMS

What is claimed is:

1. An isolated polynucleotide encoding a non-naturally occurring mutant polypeptide having acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) activity, wherein said mutant polypeptide comprises at least one mutant membrane-bound *O*-acyltransferase protein motif, and wherein the polynucleotide is operably linked to at least one regulatory sequence.

2. The isolated polynucleotide of claim 1, wherein said mutant membrane-bound *O*-acyltransferase protein motif is selected from the group consisting of:

(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 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;

(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 one amino acid 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, 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;

(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 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; and

(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 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 and 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.

3. A mutant polypeptide encoded by the isolated polynucleotide of claim 1, wherein said polypeptide has LPCAT activity.

4. A recombinant cell comprising the isolated polynucleotide of claim 1 and a polyunsaturated fatty acid biosynthetic pathway capable of producing at least one long-chain polyunsaturated fatty acid, wherein the isolated polynucleotide is over-expressed, and wherein the recombinant cell comprises at least one of the following:

(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 the amount produced by a control cell, or

(b) a C_{18} to C_{20} elongation conversion efficiency that is at least the same as the conversion efficiency of a control cell.

5. A recombinant construct comprising the isolated polynucleotide of claim 1.

6. A transformed cell comprising the recombinant construct of claim 5.

7. The transformed cell of claim 6, selected from the group consisting of microbes and plants.

8. The transformed cell of claim 7, wherein the cell is an oleaginous yeast.

9. The isolated polynucleotide of claim 1, wherein:

(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:

(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;

(ii) the amino acid mutation is 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

(iii) there are at least two amino acid mutations, wherein:

(1) a first amino acid mutation is an amino acid substitution selected from the group set forth in part (i), and

(2) a second amino acid mutation is an amino acid substitution selected from the group set forth in part (ii); and

(b) overexpression of the mutant polypeptide in a recombinant *Yarrowia* cell comprising a polyunsaturated fatty acid biosynthetic pathway capable of producing at least one long-chain polyunsaturated fatty acid produces a result selected from the group consisting of:

(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 the amount produced by a control *Yarrowia* cell, and

(ii) a C_{18} to C_{20} elongation conversion efficiency that is at least the same as the conversion efficiency of a control *Yarrowia* cell.

10. The isolated polynucleotide of claim 9, wherein the C_{18} to C_{20} elongation conversion efficiency is an effect of delta-9 elongase conversion efficiency or delta-6 elongase conversion efficiency.

11. The isolated polynucleotide of claim 9, wherein the mutant polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:37, wherein SEQ ID NO:37 differs from SEQ ID NO:4 (YILPCAT), wherein said difference is selected from the group consisting of:

(f) 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 and F390C;

(g) an amino acid mutation selected from the group consisting of: V133C, M136N, L138G, L138I, L138N, S139G, S139N, W143H, G148V, L388H, L388T, F390G, F390N and F390T;

(h) an amino acid mutation selected from the group consisting of: C135F, M136T, S140Y, S140I, F141V, G142I, G142V, D147E, F378Y, T382Y, R383A and F390S;

(i) a pair of amino acid mutations selected from the group consisting of:

(i) L134A and a second mutation selected from the group consisting of: L388G and F390T;

(ii) M136S and a second mutation selected from the group consisting of: F378Y, T382I, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C and T389S;

(iii) M136V and a second mutation selected from the group consisting of: T382P, T382Y, P384A, L388Y, T389A, T389C and T389S;

(iv) K137H and a second mutation selected from the group consisting of: P384G, L388T, F390S and F390T;

(v) K137N and a second mutation selected from the group consisting of: T382P, R383M, P384G, F378Y, L388G, L388T, T389A, T389S, F390S and F390T;

(vi) S140H and a second mutation selected from the group consisting of: P384G, L388T, F390G and F390S;

(vii) S140W and a second mutation selected from the group consisting of: T382I, T382P, T382Y, R383M, P384A, L388Y, T389A and T389C;

(viii) F141M and a second mutation selected from the group consisting of: F378Y, T382Y, R383M, P384A and T389C;

(ix) F141W and a second mutation selected from the group consisting of: F378Y, T382P, T382Y, R383M, P384A, T389A, T389C and T389S;

(x) N144A and a second mutation selected from the group consisting of: P384G, L388G, L388T, F390G, F390S and F390T;

(xi) N144T and a second mutation selected from the group consisting of: F378Y, T382P, T382Y, R383M, P384A, L388Y, T389A, T389C and T389S;

(xii) V145M and a second mutation selected from the group consisting of: T382I, R383M, T389A and T389C;

(xiii) V145W and T382I;

(xiv) D147H and a second mutation selected from the group consisting of: L388G, L388T and F390S;

(xv) D147Q and a second mutation selected from the group consisting of: T382I and F390S;

(xvi) G148A and a second mutation selected from the group consisting of: F378Y, T382I, T382Y, R383M, P384G, L388G, L388Y, T389A, T389C, F390S and F390T; and

(xvii) G148N and a second mutation selected from the group consisting of: T382I, L388T, F390G and F390S;

(j) a pair of amino acid mutations selected from the group consisting of:

(i) L134A and F390G;

(ii) L134G and a second mutation selected from the group consisting of: F390G, L388G and F390T;

(iii) K137H and a second mutation selected from the group consisting of: T382I and F390G;

(iv) S140W and T389S;

(v) F141M and T389A;

(vi) N144A and T382I;

(vii) V145W and a second mutation selected from the group consisting of: T389S and T389A;

(viii) D147Q and a second mutation selected from the group consisting of: L388T and L388G;

(ix) D147H and F390T; and

(x) G148N and P384G; and

(g) a pair of amino acid mutations selected from the group consisting of:

(i) L134A and T382I;

(ii) K137H and L388G;

(iii) K137N and a second mutation selected from the group consisting of: T389C and F390G;

(iv) S140H and a second mutation selected from the group consisting of: T382I and L388G;

(v) F141W and a second mutation selected from the group consisting of: T382I and L388Y;

(vi) F141M and T382P;

(vii) V145M and a second mutation selected from the group consisting of: T382Y and F378Y;

(viii) V145W and F378Y;

(ix) D147 and T382I;

(x) G148A and P384A;

(xi) M136S and T389A; and

(xii) G148N_F390S.

12. The isolated polynucleotide of claim 11, wherein the mutant polypeptide comprises a sequence selected from the group consisting of: SEQ ID NOs:79, 81, 83, 85, 87, 89, 91 and 93.

13. A transformed *Yarrowia* cell comprising the isolated polynucleotide of claim 9, wherein oil produced by the transformed *Yarrowia* cell comprises at least one long-chain polyunsaturated fatty acid 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.

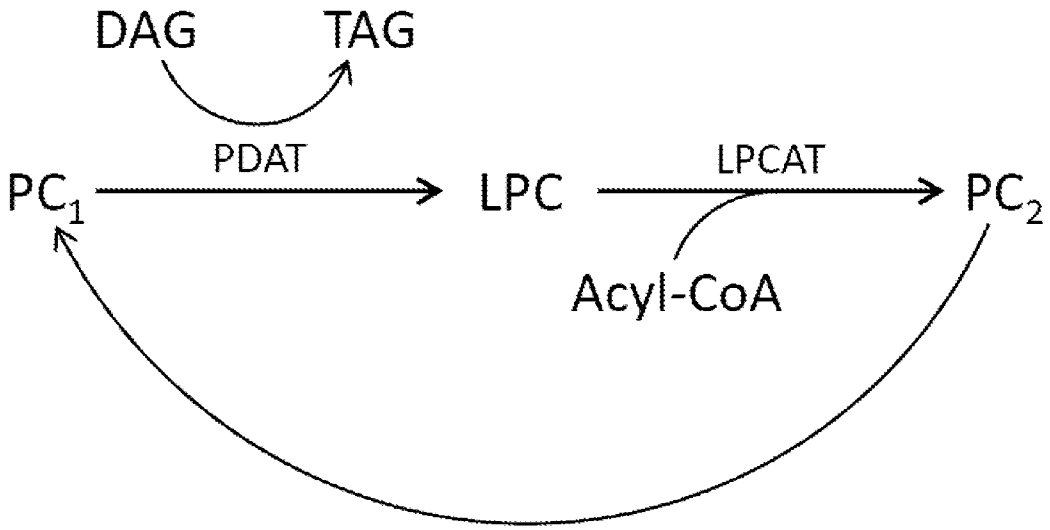


FIG. 1

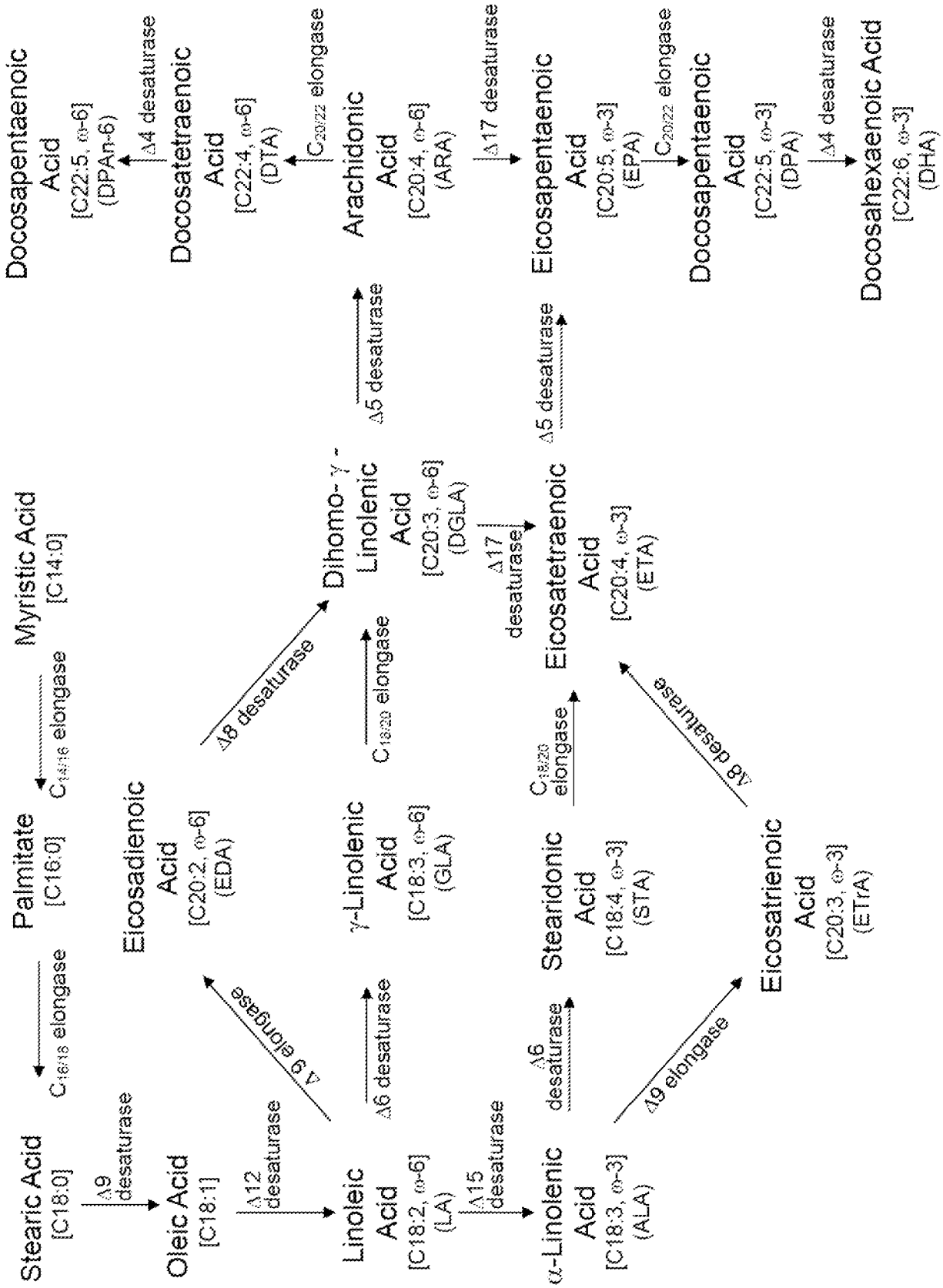
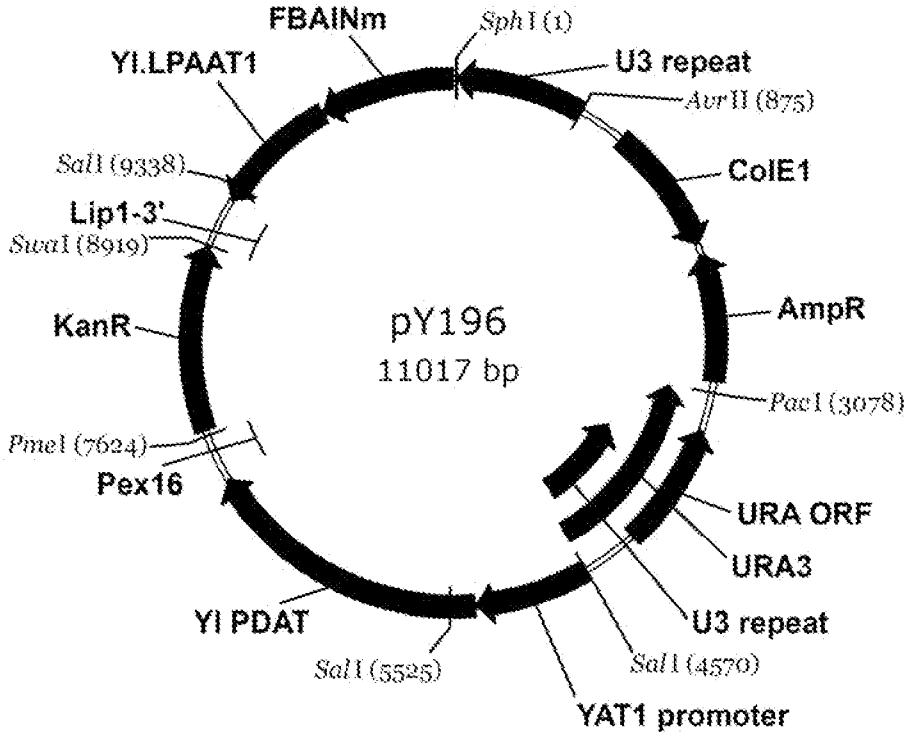

Stearic Acid
[C18:0]
Δ9 desaturase
Oleic Acid
[C18:1]
Δ12 desaturase
Linoleic Acid
[C18:2, ω-6]
(LA)
Δ15 desaturase
α-Linolenic Acid
[C18:3, ω-3]
(ALA)
C16/18 elongase
Palmitate
[C16:0]
C14/16 elongase
Myristic Acid
[C14:0]
Δ9 elongase
Eicosadienoic Acid
[C20:2, ω-6]
(EDA)
Δ6 desaturase
γ-Linolenic Acid
[C18:3, ω-6]
(GLA)
Δ8 desaturase
C18/20 elongase
Dihomo- γ - Linolenic Acid
[C20:3, ω-6]
(DGLA)
Δ6 desaturase
Stearidonic Acid
[C18:4, ω-3]
(STA)
C18/20 elongase
Eicosatetraenoic Acid
[C20:4, ω-3]
(ETA)
Δ17 desaturase
Δ9 elongase
Eicosatrienoic Acid
[C20:3, ω-3]
(ETrA)
Δ8 desaturase
Δ5 desaturase
Arachidonic Acid
[C20:4, ω-6]
(ARA)
C20/22 elongase
Docosatetraenoic Acid
[C22:4, ω-6]
(DTA)
Δ4 desaturase
Docosapentaenoic Acid
[C22:5, ω-6]
(DPAn-6)
Δ17 desaturase
Δ5 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)

FIG. 2

A)


FBAINm
Sph I (1)
Yl.LPAAT1
U3 repeat
Avr II (875)
Sal I (9338)
ColE1
Lip1-3'
Swa I (8919)
AmpR
KanR
pY196
11017 bp
Pac I (3078)
Pme I (7624)
Pex16
URA ORF
URA3
Yl PDAT
U3 repeat
Sal I (5525)
Sal I (4570)
YAT1 promoter

B)

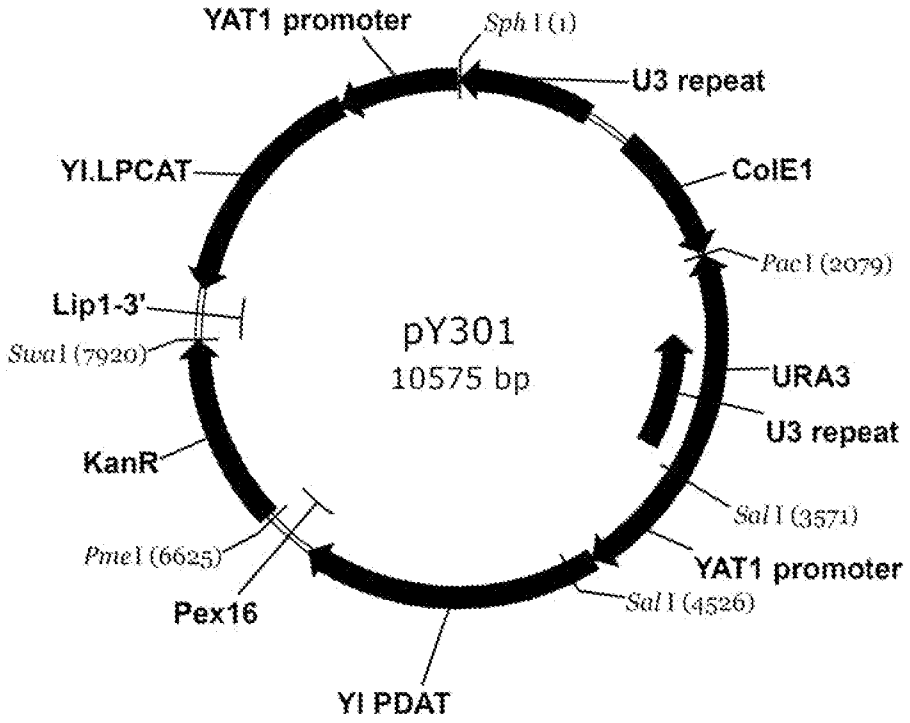

YAT1 promoter
Sph I (1)
U3 repeat
Yl.LPCAT
ColE1
Pac I (2079)
Lip1-3'
pY301
10575 bp
Swa I (7920)
URA3
U3 repeat
KanR
Sal I (3571)
Pme I (6625)
YAT1 promoter
Pex16
Sal I (4526)
Yl PDAT

FIG. 3

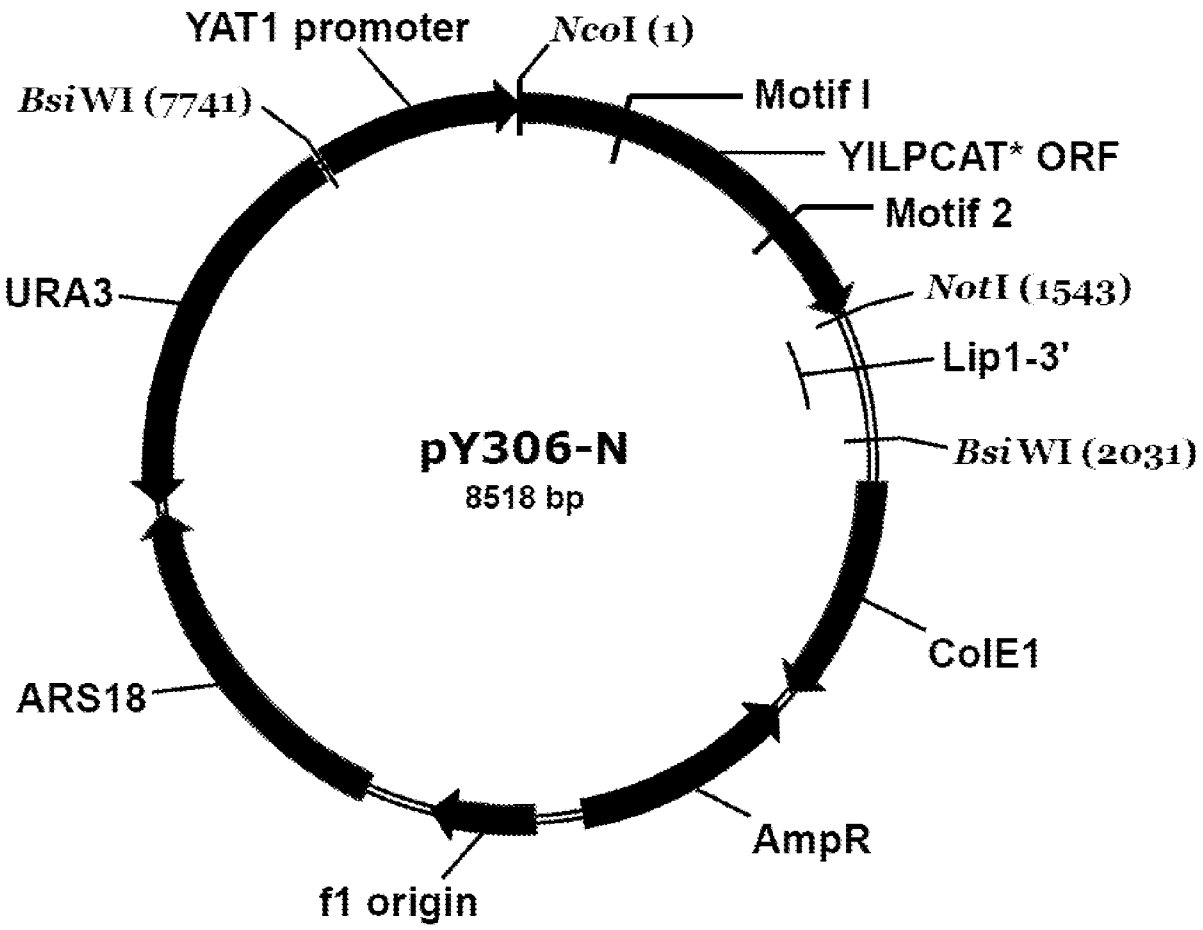

YAT1 promoter
NcoI (1)
BsiWI (7741)
Motif I
YILPCAT* ORF
Motif 2
URA3
NotI (1543)
Lip1-3'
pY306-N
8518 bp
BsiWI (2031)
ColE1
ARS18
AmpR
f1 origin

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/045628

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/10 C12P7/64
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/145867 A1 (ZOU JITAO [CA] ET AL) 19 June 2008 (2008-06-19) the whole document	1-13

A	US 2010/317882 A1 (YADAV NARENDRA S [US] ET AL) 16 December 2010 (2010-12-16) the whole document	1-13

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 2013	30/09/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Roscoe, Richard

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHINDOU H ET AL: "Identification of membrane O-acyltransferase family motifs", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 383, no. 3, 5 June 2009 (2009-06-05), pages 320-325, XP026088115, ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2009.04.013 [retrieved on 2009-04-08] the whole document	1-13
A	ERIC SOUPENE ET AL: "Phosphatidylcholine formation by LPCAT1 is regulated by Ca2+ and the redox status of the cell", BMC BIOCHEMISTRY, BIOMED CENTRAL, LONDON, GB, vol. 13, no. 1, 7 June 2012 (2012-06-07), page 8, XP021115012, ISSN: 1471-2091, DOI: 10.1186/1471-2091-13-8 the whole document	1-13
X	J. PARAMESVARAN ET AL: "Distributions of enzyme residues yielding mutants with improved substrate specificities from two different directed evolution strategies", PROTEIN ENGINEERING DESIGN AND SELECTION, vol. 22, no. 7, 1 July 2009 (2009-07-01), pages 401-411, XP55079664, ISSN: 1741-0126, DOI: 10.1093/protein/gzp020 the whole document	1-13
A	H.-C. LEE ET AL: "Caenorhabditis elegans mboa-7, a Member of the MBOAT Family, Is Required for Selective Incorporation of Polyunsaturated Fatty Acids into Phosphatidylinositol", MOLECULAR BIOLOGY OF THE CELL, vol. 19, no. 3, 27 December 2007 (2007-12-27), pages 1174-1184, XP55079614, ISSN: 1059-1524, DOI: 10.1091/mbc.E07-09-0893 Whole doc, especially Fig.2	1-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/045628

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13(partially)

   Subject-matter relating to LPCAT enzyme with modification in region MVLCMKLSSFGWNVYDG (YILPCAT) or equivalent enzymes as defined by motifs specified in claim 1, parts (a) and (b), and via specific numbered mutations in positions 132-148.

   ---

2. claims: 1-13(partially)

   Subject-matter relating to LPCAT enzyme with modification in region SAFWHGTRPGYYLTF (YILPCAT, i.e. enzyme from Yarrowia lypolytica) or equivalent enzymes as defined by motifs specified in claim 1, parts (c) and (d), and via specific numbered mutations in positions 376-390.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/045628

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008145867 A1	19-06-2008	AU 2007334364 A1	26-06-2008
		CA 2671674 A1	26-06-2008
		US 2008145867 A1	19-06-2008
		US 2010016431 A1	21-01-2010
		US 2013152230 A1	13-06-2013
		WO 2008076377 A2	26-06-2008
US 2010317882 A1	16-12-2010	US 2010317072 A1	16-12-2010
		US 2010317735 A1	16-12-2010
		US 2010317882 A1	16-12-2010