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(54) **PEROXISOME BIOGENESIS FACTOR PROTEIN (PEX) DISRUPTIONS FOR ALTERING POLYUNSATURATED FATTY ACIDS AND TOTAL LIPID CONTENT IN OLEAGINOUS EUKARYOTIC ORGANISMS**

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(51) **Int. Cl.**

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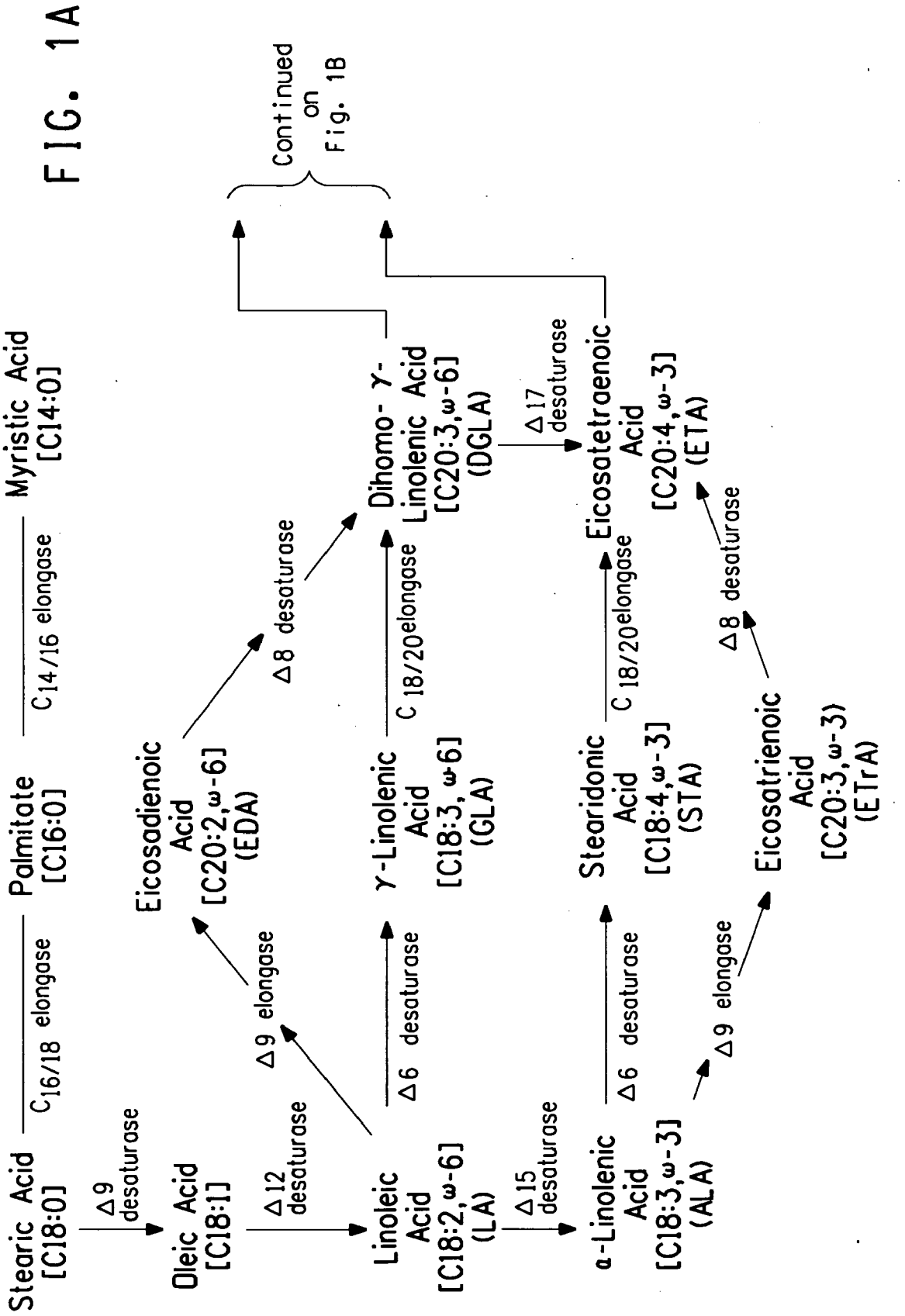
C12N 1/19	(2006.01)

(52) **U.S. Cl.** **426/601**; 435/471; 435/254.2

(57) **ABSTRACT**

Methods of increasing the amount of polyunsaturated fatty acids (PUFAs) in the total lipid fraction and in the oil fraction of PUFA-producing, oleaginous eukaryotes, accomplished by modifying the activity of peroxisome biogenesis factor (Pex) proteins. Disruptions of a chromosomal Pex3 gene, Pex10p gene or Pex16p gene in a PUFA-producing, oleaginous eukaryotic strain resulted in an increased amount of PUFAs, as a percent of total fatty acids and as a percent of dry cell weight, in the total lipid fraction and in the oil fraction of the strain, as compared to the parental strain whose native Pex protein was not disrupted.

FIG. 1A


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)
Δ6 desaturase
Stearidonic Acid [C18:4,ω-3] (STA)
Δ9 elongase
Eicosatrienoic Acid [C20:3,ω-3) (ETrA)
Δ8 desaturase
Dihomo-γ-Linolenic Acid [C20:3,ω-6] (DGLA)
C18/20elongase
Eicosatetraenoic Acid [C20:4,ω-3] (ETA)
Δ8 desaturase
Δ17 desaturase
C18/20elongase
Continued on Fig. 1B

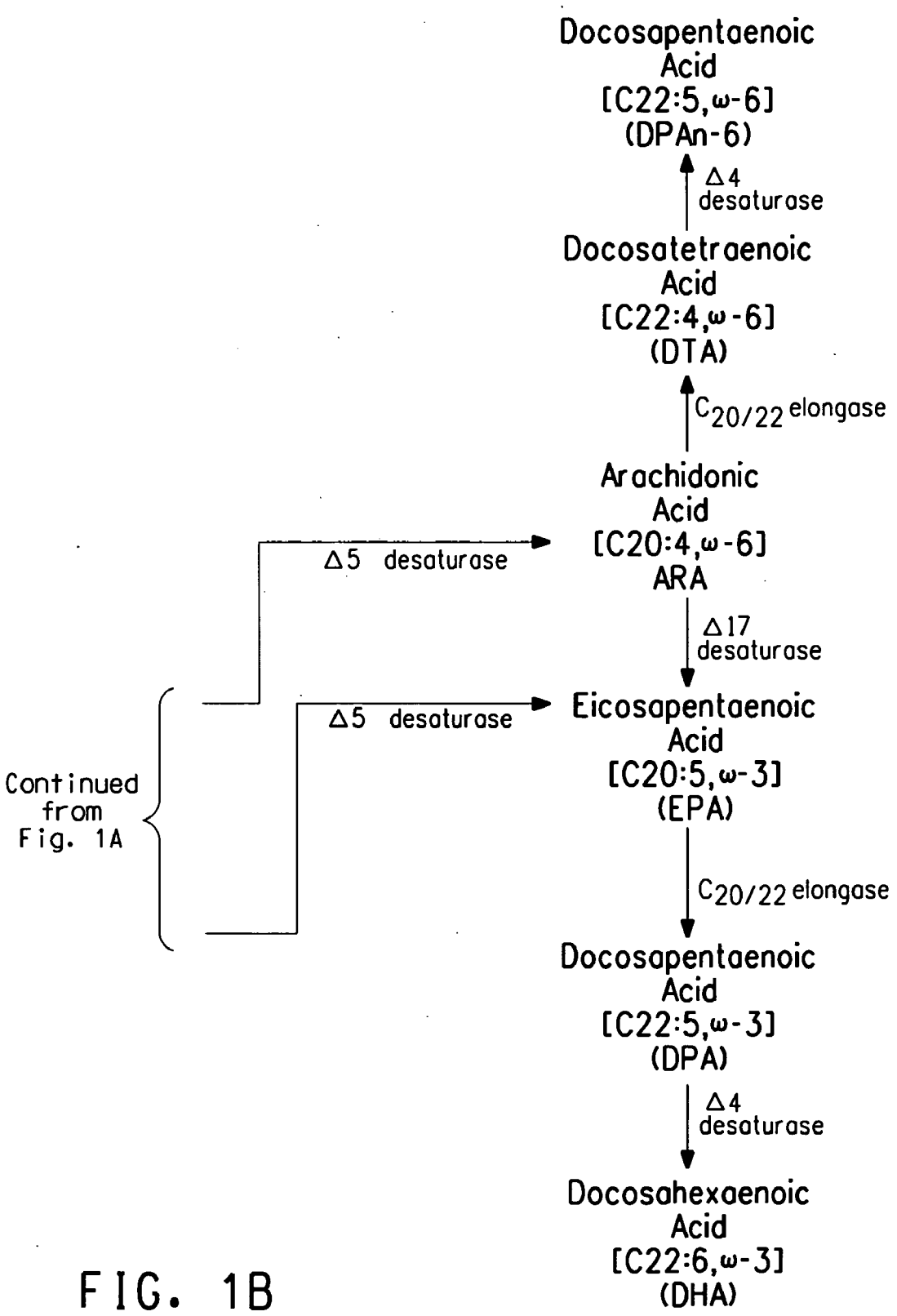

Docosapentaenoic Acid [C22:5,ω-6] (DPAn-6)
Δ4 desaturase
Docosatetraenoic Acid [C22:4,ω-6] (DTA)
C20/22 elongase
Arachidonic Acid [C20:4,ω-6] ARA
Δ5 desaturase
Δ17 desaturase
Δ5 desaturase
Eicosapentaenoic Acid [C20:5,ω-3] (EPA)
Continued from Fig. 1A
C20/22 elongase
Docosapentaenoic Acid [C22:5,ω-3] (DPA)
Δ4 desaturase
Docosahexaenoic Acid [C22:6,ω-3] (DHA)

FIG. 1B

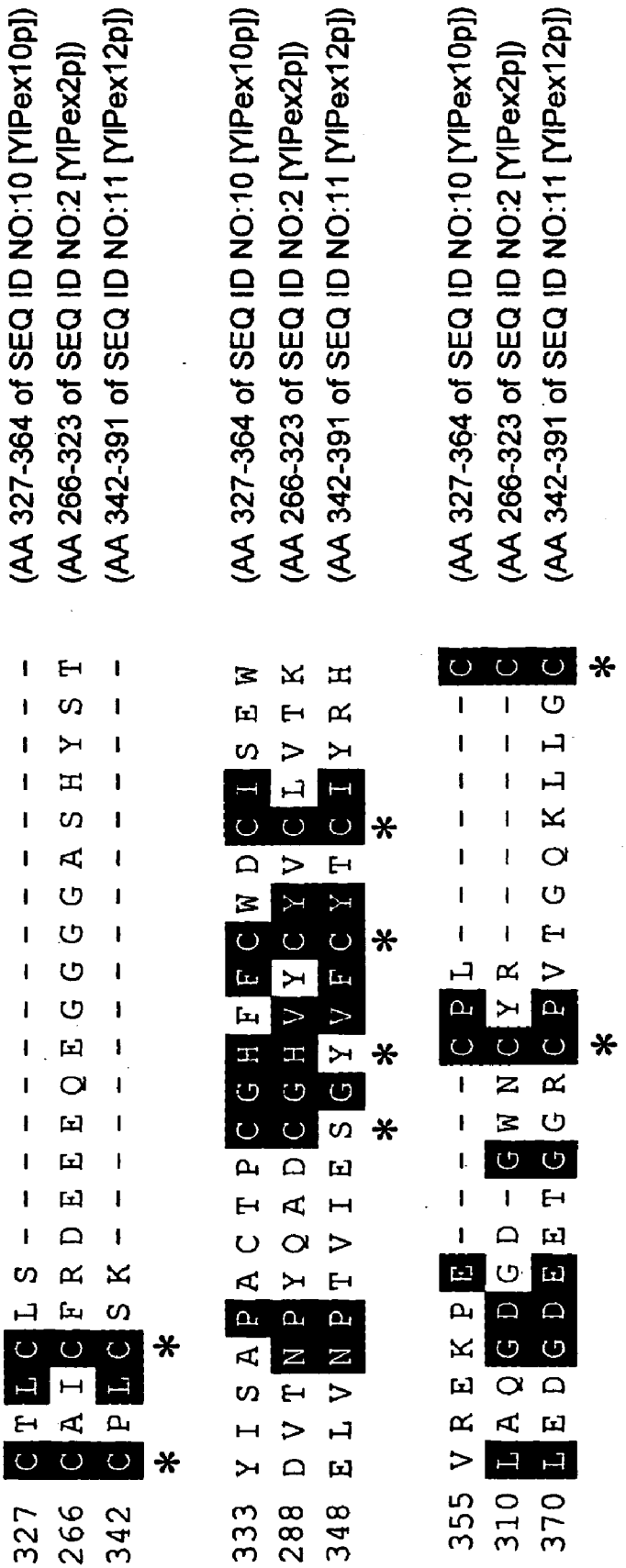

(AA 327-364 of SEQ ID NO:10 [YIPex10p])
(AA 266-323 of SEQ ID NO:2 [YIPex2p])
(AA 342-391 of SEQ ID NO:11 [YIPex12p])
(AA 327-364 of SEQ ID NO:10 [YIPex10p])
(AA 266-323 of SEQ ID NO:2 [YIPex2p])
(AA 342-391 of SEQ ID NO:11 [YIPex12p])
(AA 327-364 of SEQ ID NO:10 [YIPex10p])
(AA 266-323 of SEQ ID NO:2 [YIPex2p])
(AA 342-391 of SEQ ID NO:11 [YIPex12p])

FIG. 2A

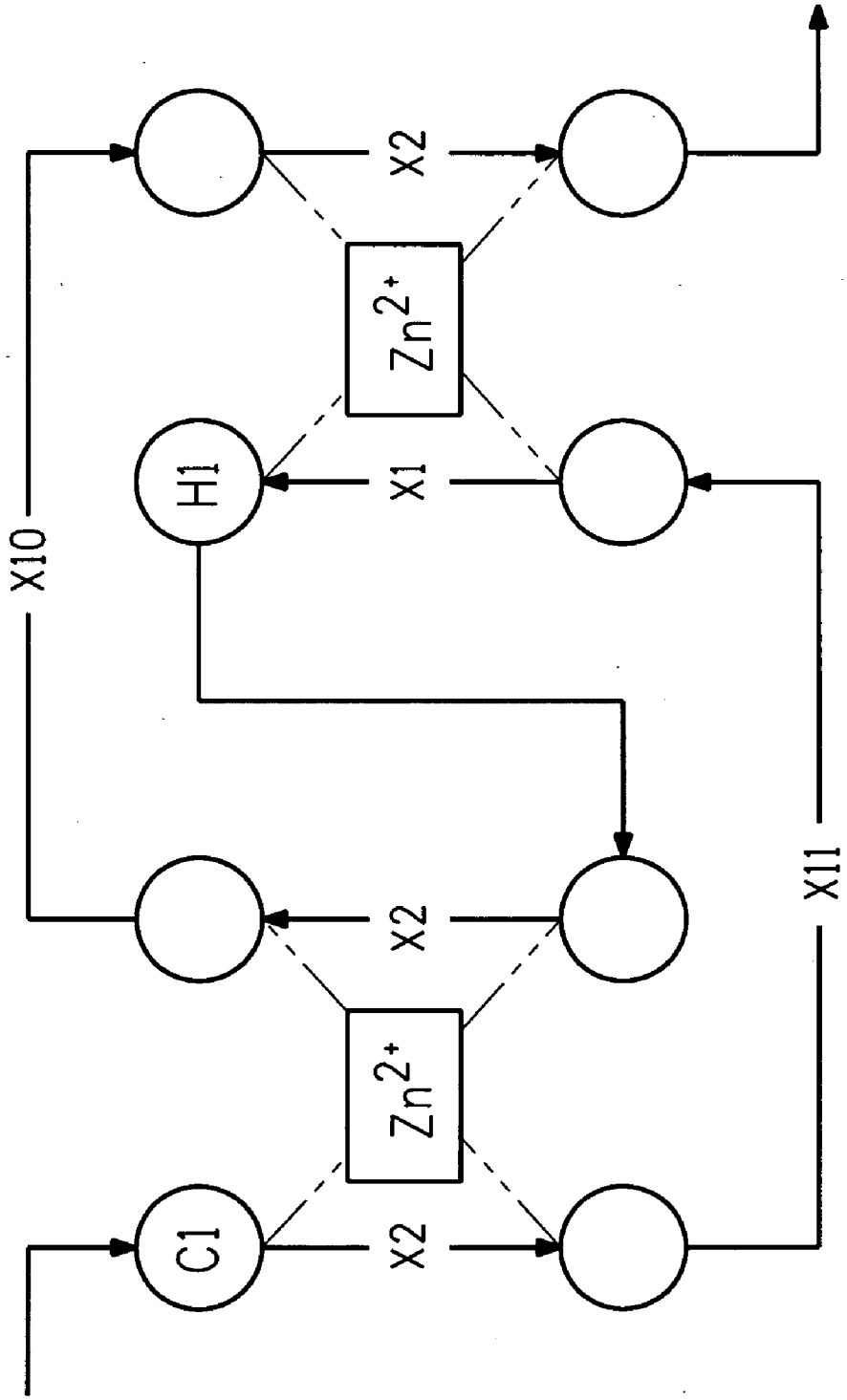


FIG. 2B

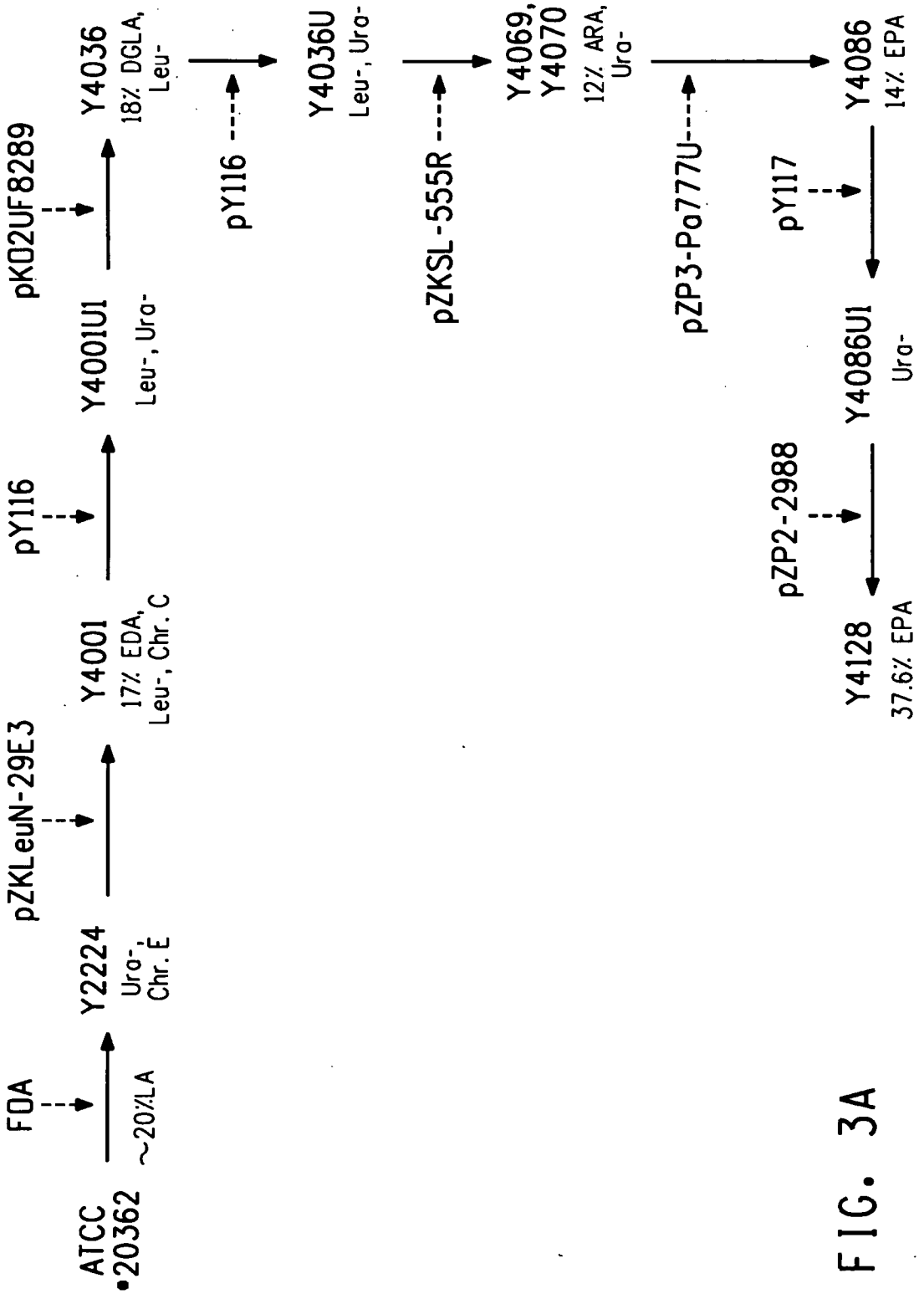

ATCC •20362
FOA
~20%LA
Y2224
Ura-; Chr.E
pZKLeuN-29E3
Y4001
17% EDA, Leu-, Chr.C
pY116
Y4001U1
Leu-, Ura-
pK02UF8289
Y4036
18% DGLA, Leu-
pY116
Y4036U
Leu-, Ura-
pZKSL-555R
Y4069, Y4070
12% ARA, Ura-
pZP3-Pa777U
pY117
Y4086
14% EPA
pZP2-2988
Y4086U1
Ura-
Y4128
37.6% EPA

FIG. 3A

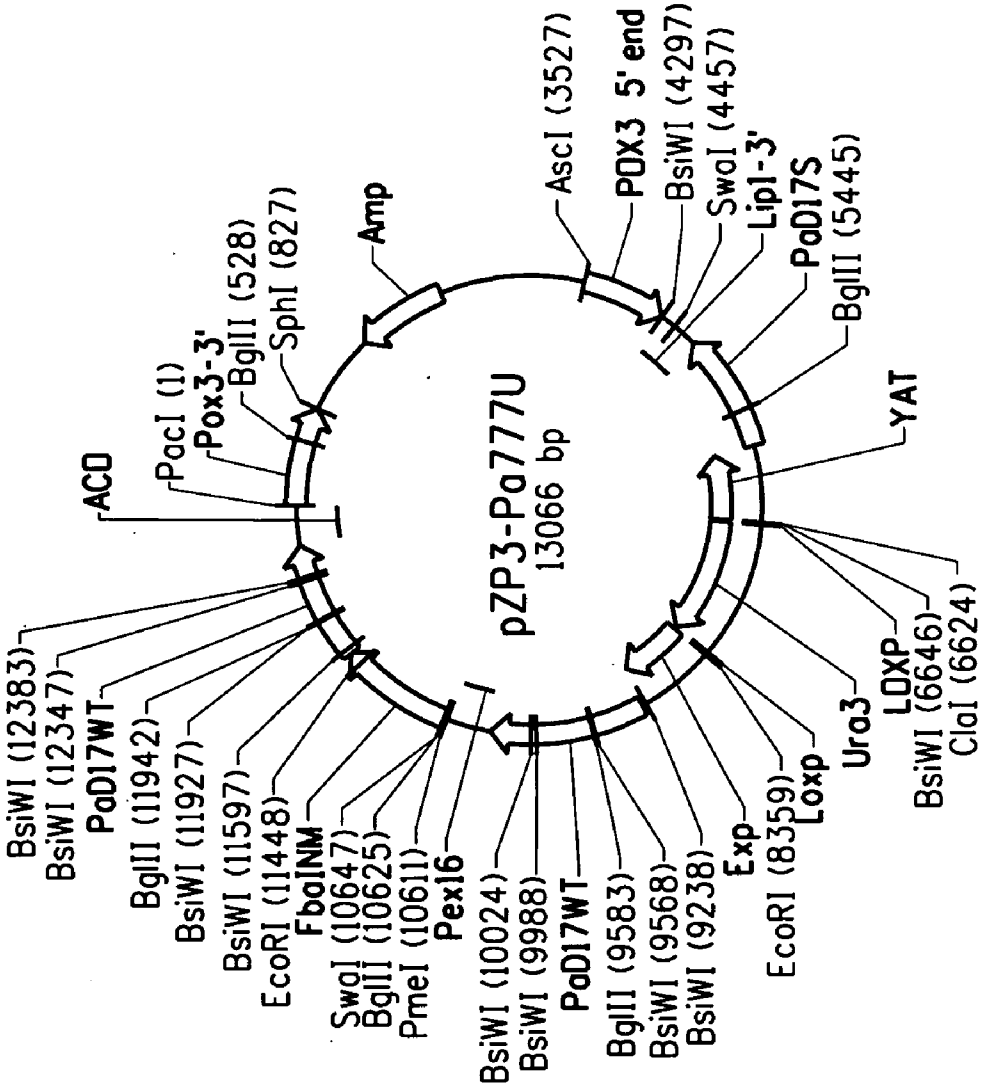

pZP3-Pa777U
13066 bp
ACO
PacI (1)
Pox3-3'
BglII (528)
SphI (827)
Amp
AscI (3527)
POX3 5' end
BsiWI (4297)
SwaI (4457)
Lip1-3'
PaD17S
BglII (5445)
YAT
Ura3
LOXP
BsiWI (6646)
ClaI (6624)
EcoRI (8359)
Loxp
Exp
BsiWI (9238)
BsiWI (9568)
BglII (9583)
PaD17WT
BsiWI (9988)
BsiWI (10024)
Pex16
PmeI (10611)
BglII (10625)
SwaI (10647)
FbaINM
EcoRI (11448)
BsiWI (11597)
BsiWI (11927)
BglII (11942)
PaD17WT
BsiWI (12347)
BsiWI (12383)

FIG. 3B

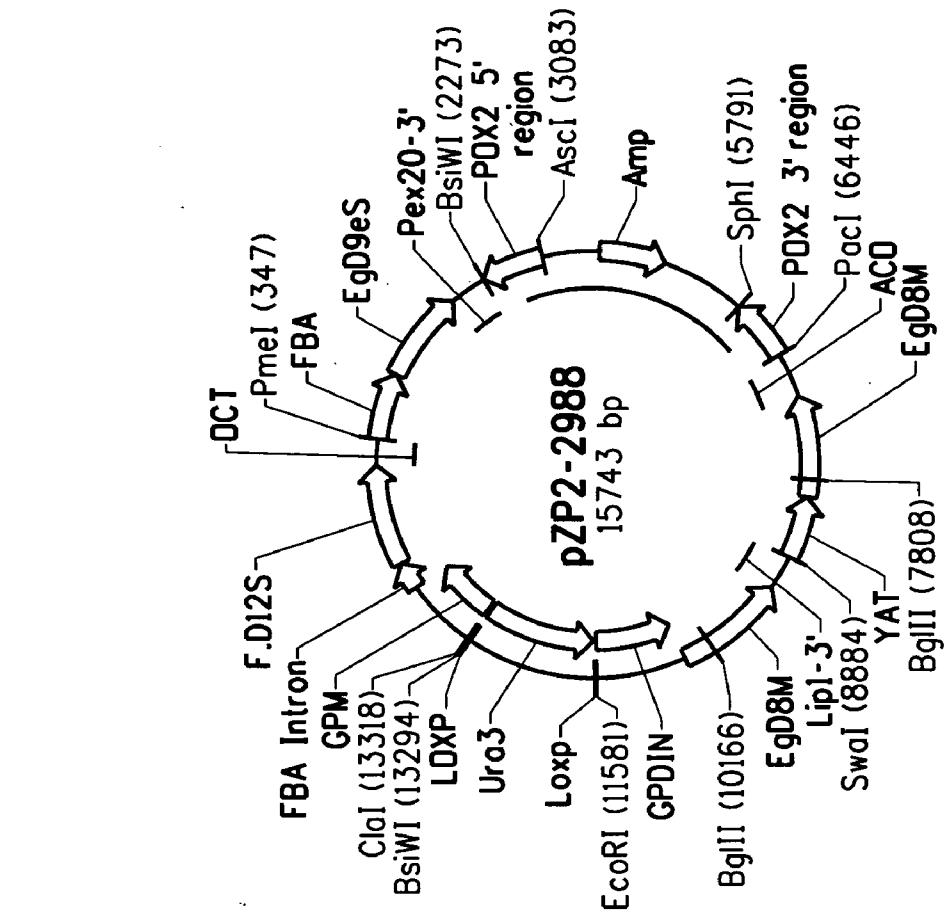

pZP2-2988
15743 bp
DCT
PmeI (347)
FBA
EgD9eS
Pex20-3'
BsiWI (2273)
POX2 5' region
AscI (3083)
Amp
SphI (5791)
POX2 3' region
PacI (6446)
ACO
EgD8M
BglII (7808)
YAT
SwaI (8884)
Lip1-3'
EgD8M
BglII (10166)
GPDIN
EcoRI (11581)
Loxp
Ura3
LOXP
BsiWI (13294)
ClaI (13318)
GPM
FBA Intron
F.D12S

FIG. 4B

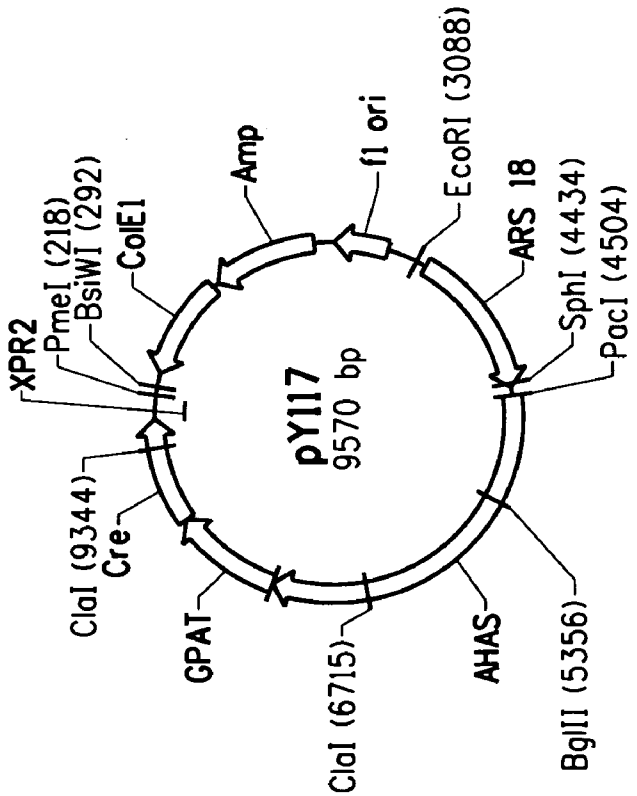

pY117
9570 bp
XPR2
PmeI (218)
BsiWI (292)
ColE1
Amp
f1 ori
EcoRI (3088)
ARS 18
SphI (4434)
PacI (4504)
ClaI (9344)
Cre
GPAT
ClaI (6715)
AHAS
BglII (5356)

FIG. 4A

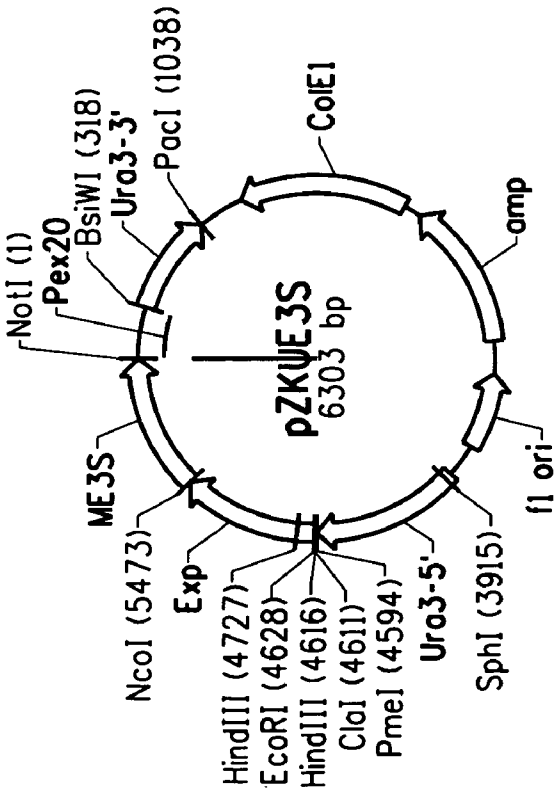

pZKΨE3S
6303 bp
NotI (1)
Pex20
BsiWI (318)
Ura3-3'
PacI (1038)
ColE1
amp
f1 ori
SphI (3915)
Ura3-5'
ClaI (4611)
PmeI (4594)
HindIII (4616)
EcoRI (4628)
HindIII (4727)
Exp
NcoI (5473)
ME3S

FIG. 5A

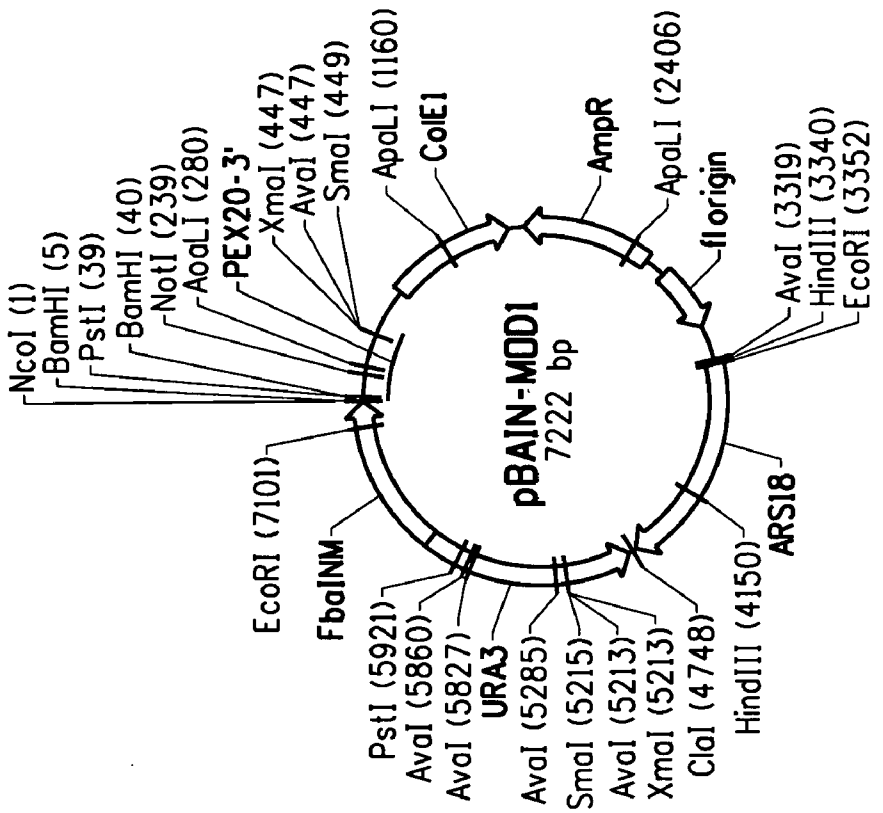

pBAIN-MOD1
7222 bp
NcoI (1)
BamHI (5)
PstI (39)
NotI (239)
AoaLI (280)
PEX20-3'
XmaI (447)
AvaI (447)
SmaI (449)
ApaLI (1160)
ColE1
AmpR
ApaLI (2406)
f1 origin
AvaI (3319)
HindIII (3340)
EcoRI (3352)
ARS18
HindIII (4150)
ClaI (4748)
XmaI (5213)
AvaI (5213)
SmaI (5215)
AvaI (5285)
URA3
AvaI (5827)
AvaI (5860)
PstI (5921)
FbaINM
EcoRI (7101)

FIG. 5B

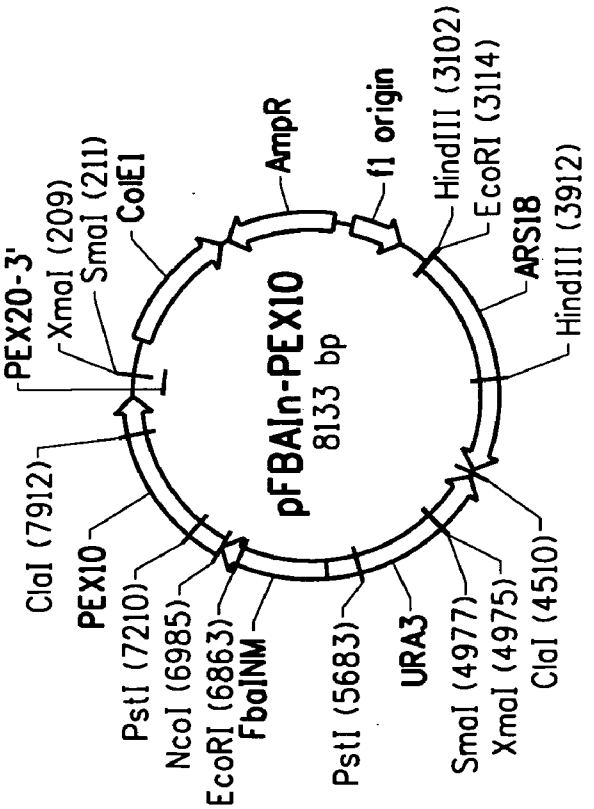

pFBAIn-PEX10
8133 bp
PEX20-3'
XmaI (209)
SmaI (211)
ColE1
AmpR
f1 origin
HindIII (3102)
EcoRI (3114)
ARS18
HindIII (3912)
ClaI (4510)
SmaI (4975)
XmaI (4977)
URA3
PstI (5683)
FbaINM
EcoRI (6863)
NcoI (6985)
PstI (7210)
PEX10
ClaI (7912)

FIG. 6A

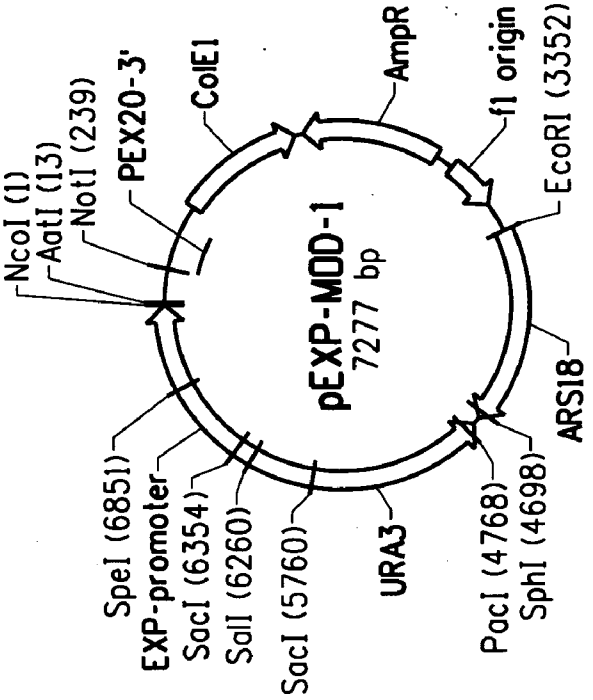

pEXP-MOD-1
7277 bp
NcoI (1)
AatI (13)
NotI (239)
PEX20-3'
ColE1
AmpR
f1 origin
EcoRI (3352)
ARS18
PacI (4768)
SphI (4698)
URA3
SacI (5760)
SalI (6260)
SacI (6354)
EXP-promoter
SpeI (6851)

FIG. 6B

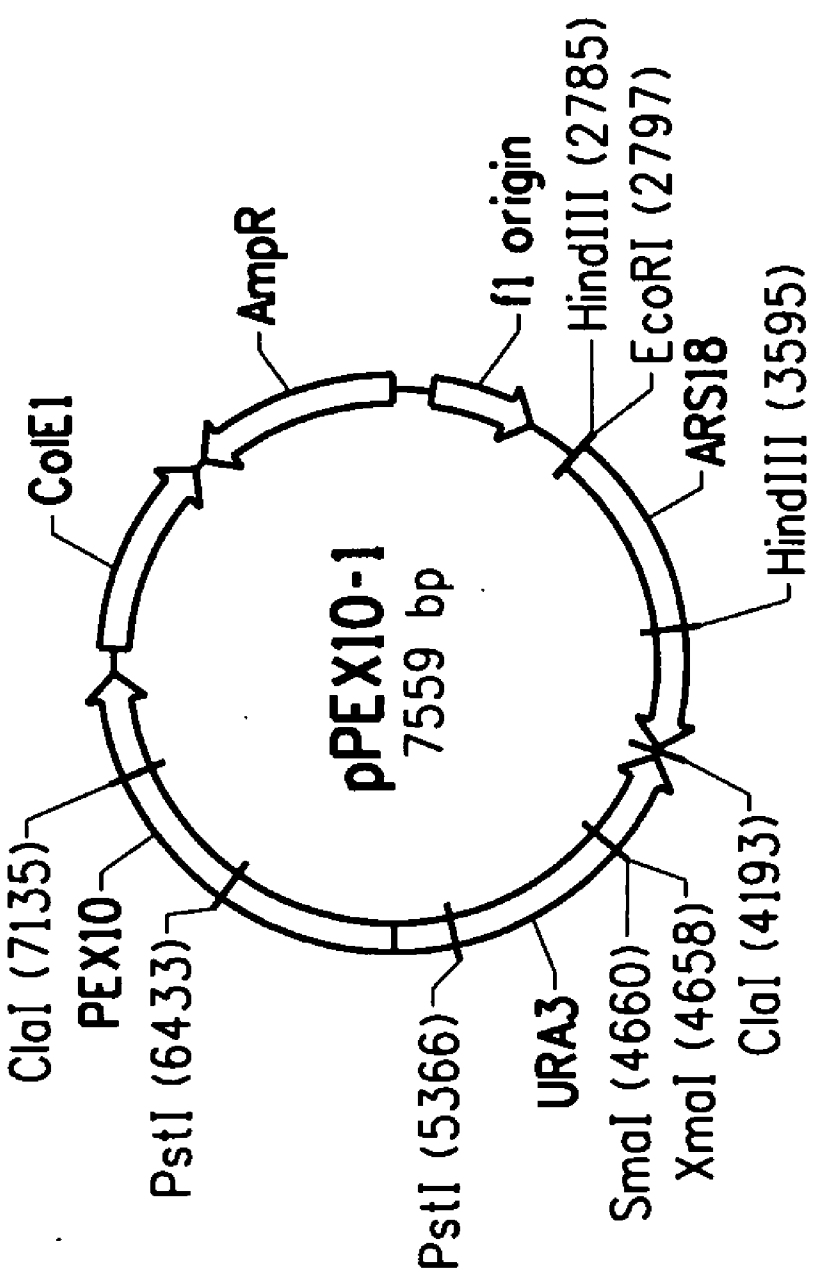

pPEX10-1
7559 bp
AmpR
f1 origin
HindIII (2785)
EcoRI (2797)
ARS18
HindIII (3595)
ClaI (4193)
XmaI (4658)
SmaI (4660)
URA3
PstI (5366)
PstI (6433)
PEX10
ClaI (7135)
ColE1

FIG. 7A


Y4069
pZP3-Pa777U
12% ARA, Ura-
Y4084
pY117
14% EPA
Y4084U1
Ura-
pZP2-2988
Y4127
18% EPA
pZKUE3S
Y4127U2
Ura-
pZKL1-2SP98C
Y4158
25% EPA
pZKUE3S
Y4184U
Ura-
Y4184
30.7% EPA
pZKUE3S
Y4184U1
Ura-
pZKL2-5U89GC

FIG. 7B

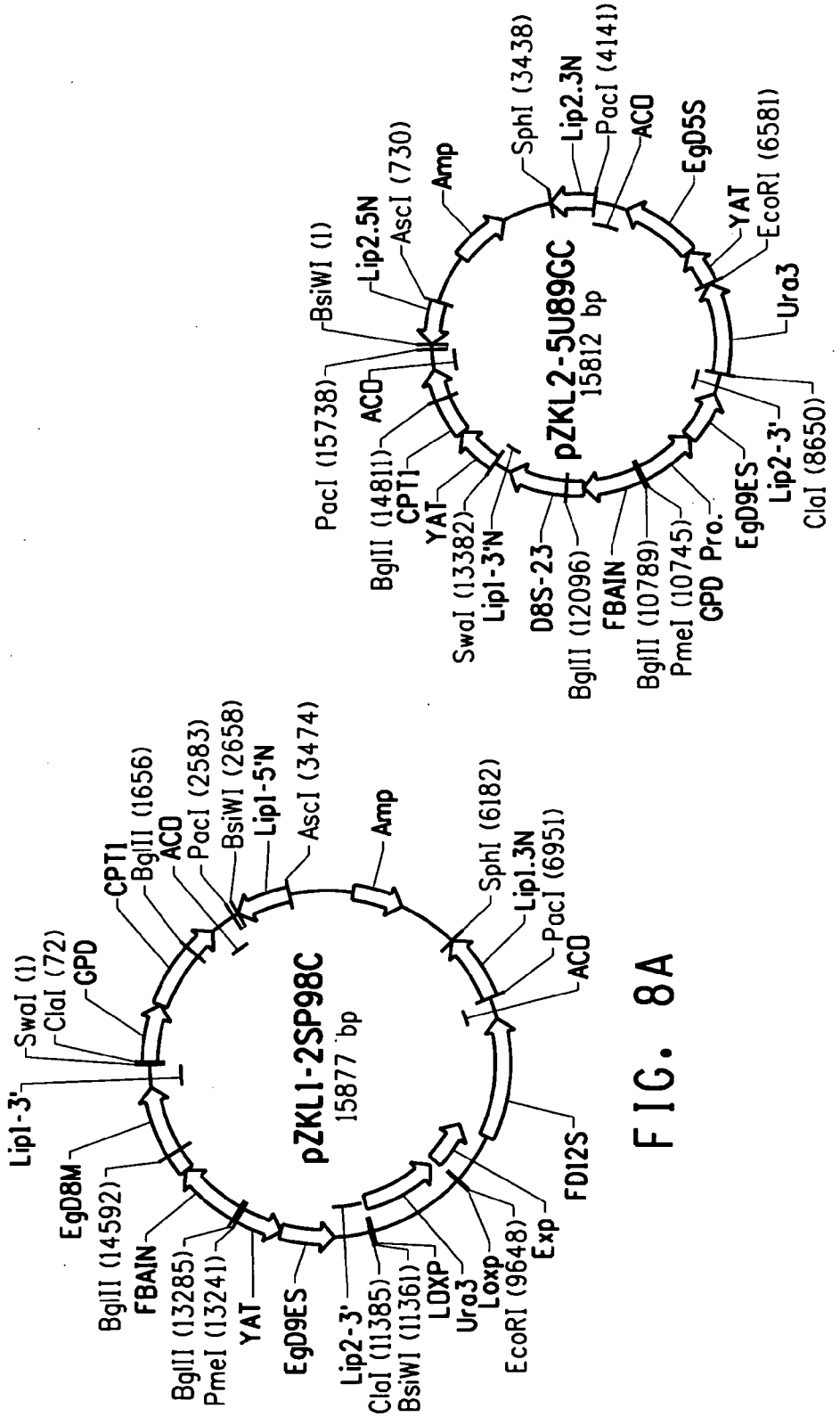

pZKL1-2SP98C
15877 bp
SwaI (1)
ClaI (72)
GPD
CPT1
BglII (1656)
ACO
PacI (2583)
BsiWI (2658)
Lip1-5'N
AscI (3474)
Amp
SphI (6182)
Lip1.3N
PacI (6951)
ACO
FD12S
Exp
EcoRI (9648)
Loxp
Ura3
LOXP
BsiWI (11361)
ClaI (11385)
Lip2-3'
EgD9ES
YAT
PmeI (13241)
BglII (13285)
FBAIN
BglII (14592)
EgD8M
Lip1-3'
pZKL2-5U89GC
15812 bp
BsiWI (1)
Lip2.5N
AscI (730)
Amp
SphI (3438)
Lip2.3N
PacI (4141)
ACO
EgD5S
YAT
EcoRI (6581)
Ura3
Lip2-3'
ClaI (8650)
EgD9ES
GPD Pro.
PmeI (10745)
BglII (10789)
FBAIN
BglII (12096)
D8S-23
Lip1-3'N
SwaI (13382)
YAT
CPT1
BglII (14811)
ACO
PacI (15738)

FIG. 8A

FIG. 8B

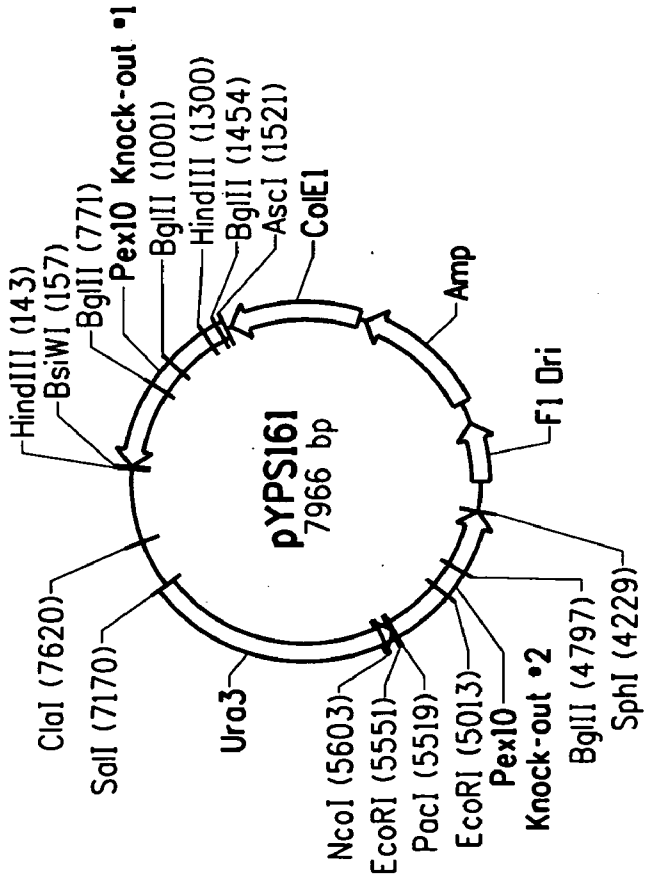

pYPS161
7966 bp
HindIII (143)
BsiWI (157)
BglII (771)
Pex10 Knock-out •1
BglII (1001)
HindIII (1300)
BglII (1454)
AscI (1521)
ColE1
Amp
F1 Ori
BglII (4797)
SphI (4229)
Knock-out •2
Pex10
EcoRI (5013)
PacI (5519)
EcoRI (5551)
NcoI (5603)
Ura3
SalI (7170)
ClaI (7620)

FIG. 9A

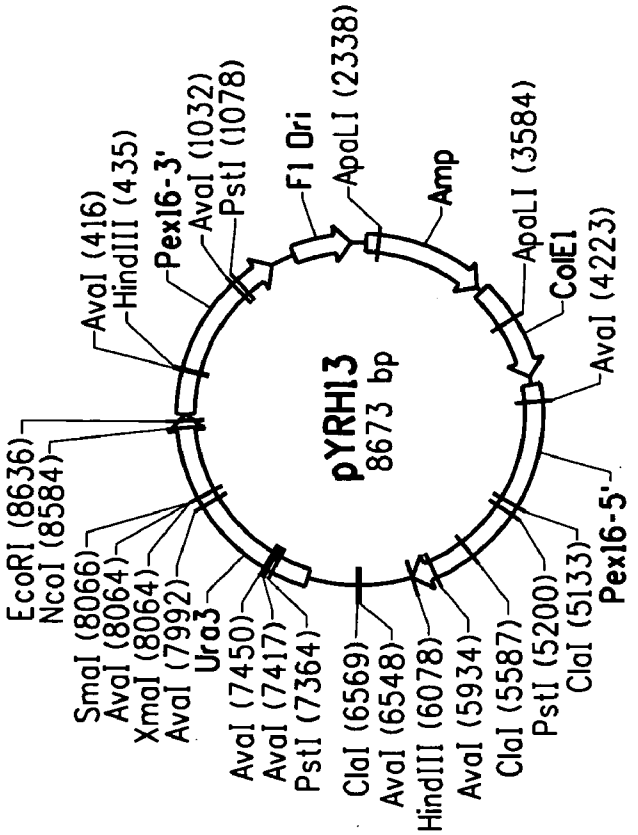

pYRH13
8673 bp
AvaI (416)
HindIII (435)
Pex16-3'
AvaI (1032)
PstI (1078)
F1 Ori
ApaLI (2338)
Amp
ApaLI (3584)
ColE1
AvaI (4223)
Pex16-5'
ClaI (5133)
PstI (5200)
ClaI (5587)
AvaI (5934)
HindIII (6078)
AvaI (6548)
ClaI (6569)
PstI (7364)
AvaI (7417)
AvaI (7450)
Ura3
AvaI (7992)
XmaI (8064)
AvaI (8064)
SmaI (8066)
NcoI (8584)
EcoRI (8636)

FIG. 9B

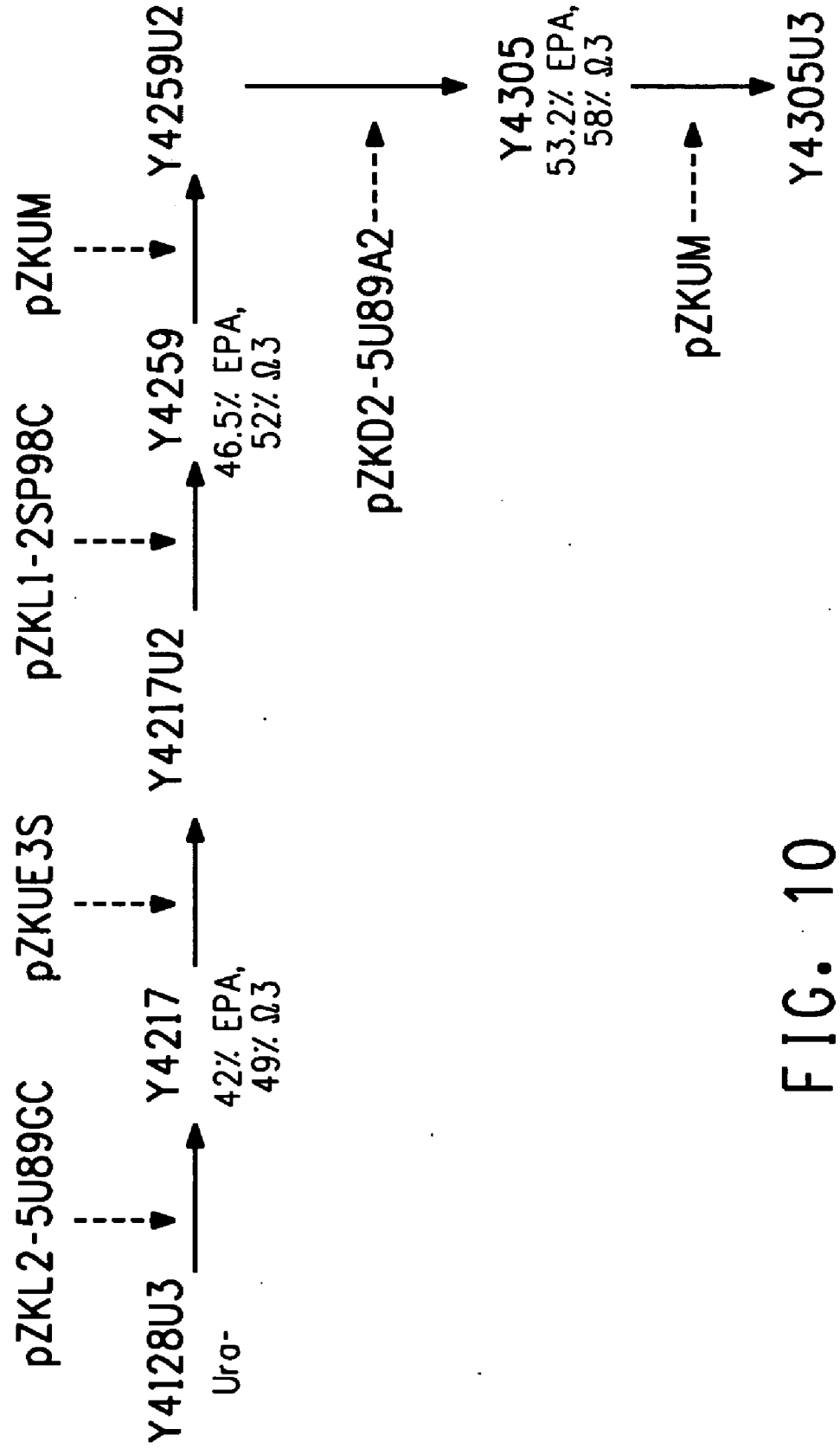

Y4128U3
Ura-
pZKL2-5U89GC
Y4217
42% EPA,
49% Ω3
pZKUE3S
Y4217U2
pZKL1-2SP98C
Y4259
46.5% EPA,
52% Ω3
pZKUM
Y4259U2
pZKD2-5U89A2----
Y4305
53.2% EPA,
58% Ω3
pZKUM----
Y4305U3

FIG. 10

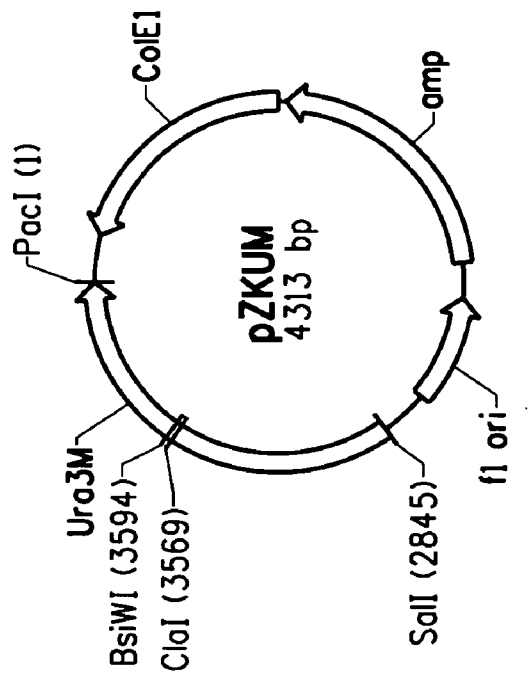


FIG. 11A

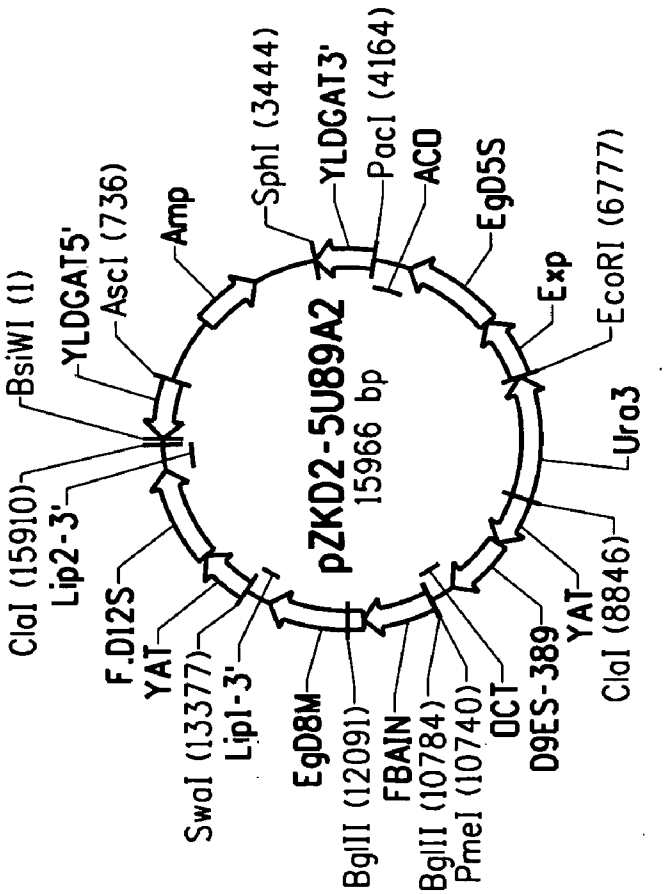


FIG. 11B

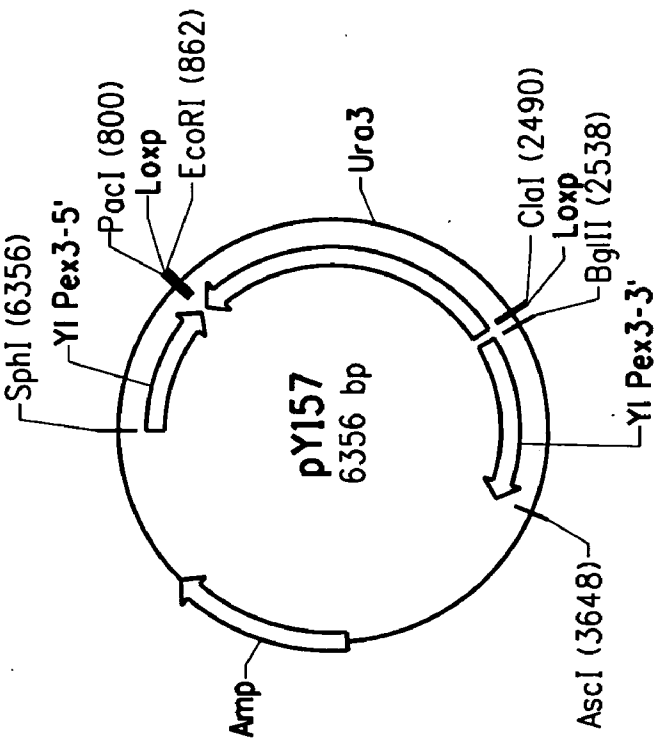


FIG. 12B

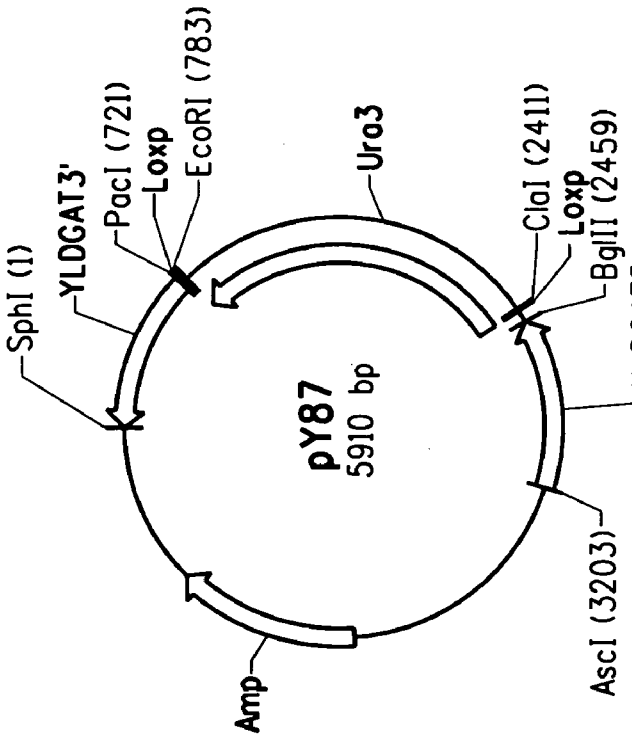


FIG. 12A

PEROXISOME BIOGENESIS FACTOR PROTEIN (PEX) DISRUPTIONS FOR ALTERING POLYUNSATURATED FATTY ACIDS AND TOTAL LIPID CONTENT IN OLEAGINOUS EUKARYOTIC ORGANISMS

[0001] This application claims the benefit of U.S. Provisional Applications No. 60/977,174 and No. 60/977,177, both filed Oct. 3, 2007 and both hereby incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention is in the field of biotechnology. More specifically, this invention pertains to methods useful for manipulating the polyunsaturated fatty acid (PUFA) composition and lipid content of eukaryotic organisms, based on disruption of peroxisome biogenesis factor (Pex) proteins.

BACKGROUND OF THE INVENTION

[0003] The health benefits associated with polyunsaturated fatty acids ["PUFAs"], especially ω-3 and ω-6 PUFAs, have been well documented. In order to find ways to produce large-scale quantities of ω-3 and ω-6 PUFAs, researchers have directed their work toward the discovery of genes and the understanding of the encoded biosynthetic pathways that result in lipids and fatty acids.

[0004] One effort to produce these PUFAs has introduced ω-3/ω-6 PUFA biosynthetic pathways into organisms that do not natively produce ω-3/ω-6 PUFAs. One such organism that has been extensively manipulated is the non-oleaginous yeast, *Saccharomyces cerevisiae*. However, none of the preliminary results demonstrating limited production of linoleic acid ["LA"], γ-linolenic acid ["GLA"], α-linolenic acid ["ALA"], stearidonic acid ["STA"] and/or eicosapentaenoic acid ["EPA"] are suitable for commercial exploitation.

[0005] Other efforts to produce large-scale quantities of ω-3/ω-6 PUFAs have cultivated microbial organisms that natively produce the fatty acid of choice, e.g., heterotrophic diatoms *Cyclotella* sp. and *Nitzschia* sp., *Pseudomonas, Alteromonas* or *Shewanella* species, filamentous fungi of the genus *Pythium*, or *Mortierella elongata, M. exigua* or *M. hygrophila.*

[0006] All these efforts suffer from an inability to substantially improve the yield of oil or to control the characteristics of the oil composition produced, since the fermentations rely on the natural abilities of the microbes themselves.

[0007] Commonly owned U.S. Pat. No. 7,238,482 describes the use of the oleaginous yeast *Yarrowia lipolytica* as a production host for the production of PUFAs. Oleaginous yeast are defined as those yeast that are naturally capable of oil synthesis and accumulation, where greater than 25% of the cellular dry weight is typical. Optimization of the production host has been described in the art (see for example Int'l. App. Pub. No. WO 2006/033723, U.S. Pat. App. Pub. No. 2006-0094092, U.S. Pat. App. Pub. No. 2006-0115881, and U.S. Pat. App. Pub. No. 2006-0110806). The recombinant strains described therein comprise various chimeric genes expressing multiple copies of heterologous desaturases, elongases and acyltransferases and optionally comprise various native desaturase and acyltransferase knockouts to enable PUFA synthesis and accumulation. Further optimization of the host cell is needed for commercial production of PUFAs.

[0008] Lin Y. et al suggest that peroxisomes are required for both catabolic and anabolic lipid metabolism (*Plant Physiology,* 135:814-827 (2004)). However, this hypothesis was based on studies with a homolog of Pex16p in *Arabidopsis* mutants that had both abnormal peroxisome biogenesis and fatty acid synthesis (i.e., a reduction of oil to approximately 10-16% of wild type in sse1 seeds was reported). Binns, D. et al. (*J. Cell Biol.,* 173(5):719-731 (2006)) also document an intimate collaboration between peroxisomes and lipid bodies in *Saccharomyces cerevisiae*. But, previous studies of Pex knockouts have not been performed in a PUFA-producing organism.

[0009] Applicants have solved the stated problem of optimizing host cells for commercial production of PUFAs by the unpredictable mechanism of disruption of peroxisome biogenesis factor proteins in a PUFA-producing organism, which leads to the unpredictable result of an increase in the amount of PUFAs, as a percent of total fatty acids, in a recombinant PUFA-producing strain of *Y. lipolytica*. Novel strains containing disruptions in peroxisome biogenesis factor proteins are described herein.

SUMMARY OF THE INVENTION

[0010] Described herein are methods of increasing the weight percent of at least one polyunsaturated fatty acid ["PUFA"] relative to the weight percent of total fatty acids ["TFAs"] in an oleaginous eukaryotic organism having a total lipid content, a total lipid fraction and an oil fraction, comprising:

a) providing an oleaginous eukaryotic organism comprising:

[0011] 1) genes encoding a functional polyunsaturated fatty acid biosynthetic pathway; and

[0012] 2) a disruption in a native gene encoding a peroxisome biogenesis factor protein, thereby providing a PEX-disrupted organism, and

b) growing the PEX-disrupted organism under conditions as to increase the weight percent of at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids in the total lipid fraction or in the oil fraction, when compared to the weight percent of the at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids in the total lipid fraction or in the oil fraction in the oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

[0013] This method of increasing may also be used to increase the percent of at least one polyunsaturated fatty acid ["PUFA"] relative to the dry cell weight ["DCW"] by applying the same steps (a) and (b).

[0014] In some of the methods described here, the weight percent of the PUFA relative to the weight percent of the TFAs is increased at least 1.3 fold.

[0015] In some of the described methods, the total lipid content in the PEX-disrupted organism may be increased or decreased compared with that of an oleaginous eukaryote having no disruption in a native PEX gene.

[0016] In any of these methods, the increased PUFA may be a single PUFA or a combination of PUFAs. In either case, the increased PUFA or increased combination of PUFAs can include linoleic acid, conjugated linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, docosatetraenoic acid, ω-6 docosapentaenoic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, ω-3 docosapentaenoic acid, eicosadienoic acid, eicosatrienoic acid, docosahexaenoic acid, hydroxylated or epoxy

fatty acids of these, a C_{18} polyunsaturated fatty acid or a combination of these, a C_{20} polyunsaturated fatty acid or a combination of these, a combination of C_{20-22} polyunsaturated fatty acids and a C_{22} polyunsaturated fatty acid or a combination of these.

[0017] In any of these methods, the PEX-disrupted organism may be a member of the following: *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon, Lipomyces, Mortierella Thraustochytrium, Schizochytrium*, and *Saccharomyces* having the property of oleaginy. And, in any of the described methods, the PUFA biosynthetic pathway includes genes that encodes any or a combination of the following enzymes: Δ9 desaturase, Δ12 desaturase, Δ6 desaturase, Δ5 desaturase, Δ17 desaturase, Δ8 desaturase, Δ15 desaturase, Δ4 desaturase, $C_{14/16}$ elongase, $C_{16/18}$ elongase, $C_{18/20}$ elongase, $C_{20/22}$ elongase and Δ9 elongase.

[0018] The disruption may occur in a PEX gene that encodes a peroxisome biogenesis factor protein that includes the following: Pex1p, Pex 2p, Pex3p, Pex3Bp, Pex4p, Pex5p, Pex5Bp, Pex5Cp, Pex5/20p, Pex6p, Pex7p, Pex8p, Pex10p, Pex12p, Pex13p, Pex14p, Pex15p, Pex16p, Pex17p, Pex14/17p, Pex18p, Pex19p, Pex20p, Pex21p, Pex21Bp, Pex22p, Pex22p-like and Pex26p. And in any of these methods, the disruption may be a gene knockout or a deletion in a portion of the gene that encodes the C-terminal portion of the protein. In some of these methods, the deletion is in the portion of the gene encoding the C-terminal portion of the C_3HC_4 zinc ring finger motif of the protein.

[0019] Also described herein is the oil fraction or the total lipid fraction in a PEX-disrupted organism, which has experienced an increase in the weight percent of at least one PUFA accomplished by the method of Claim **1**. Described herein is also a PEX-disrupted *Yarrowia lipolytica*, having a disruption in a native gene encoding Pex3p or Pex10p or Pex16p. This *Y. lipolytica* may have ATCC designation ATCC PTA-8614 (strain Y4128).

Biological Deposits

[0020] The following biological materials have been deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, and bear the following designations, accession numbers and dates of deposit.

Biological Material	Accession No.	Date of Deposit
Yarrowia lipolytica Y2047	ATCC PTA-7186	Oct. 26, 2005
Yarrowia lipolytica Y2201	ATCC PTA-7185	Oct. 26, 2005
Yarrowia lipolytica Y2096	ATCC PTA-7184	Oct. 26, 2005
Yarrowia lipolytica Y3000	ATCC PTA-7187	Oct. 26, 2005
Yarrowia lipolytica Y4128	ATCC PTA-8614	Aug. 23, 2007
Yarrowia lipolytica Y4127	ATCC PTA-8802	Nov. 29, 2007

The biological materials listed above were deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The listed deposit will be maintained in the indicated international depository for at least 30 years and will be made available to the public upon the grant of a patent disclosing it. The availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by government action.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTINGS

[0021] FIG. **1** consists of FIG. **1**A and FIG. **1**B, which together illustrate the ω-3/ω-6 fatty acid biosynthetic pathway, and should be viewed together when considering the description of this pathway below.

[0022] FIG. **2**A provides an alignment of the C_3HC_4 zinc ring finger motifs of the *Yarrowia lipolytica* Pex10p (i.e., amino acids 327-364 of SEQ ID NO:10 [GenBank Accession No. CAG81606]), the *Yarrowia lipolytica* Pex2p (i.e., amino acids 266-323 of SEQ ID NO:2 [GenBank Accession No. CAG77647]) and the *Yarrowia lipolytica* Pex12p (i.e., amino acids 342-391 of SEQ ID NO:11 [GenBank Accession No. CAG81532]), with cysteine and histidine residues of the conserved C_3HC_4 zinc ring finger motif indicated by asterisks.

[0023] FIG. **2**B schematically illustrates the proposed interaction between various amino acid residues of the *Y. lipolytica* Pex10p C_3HC_4 finger motif and the two zinc ions to which they bind.

[0024] FIG. **3**A diagrams the development of *Yarrowia lipolytica* strain Y4128, producing 37.6% EPA in the total lipid fraction.

[0025] FIG. **3**B provides a plasmid map for pZP3-Pa777U.

[0026] FIG. **4** provides plasmid maps for the following: (A) pY117; and, (B) pZP2-2988.

[0027] FIG. **5** provides plasmid maps for the following: (A) pZKUE3S; and, (B) pFBAIN-MOD-1.

[0028] FIG. **6** provides plasmid maps for the following: (A) pFBAIN-PEX10; and, (B) pEXP-MOD-1.

[0029] FIG. **7**A provides a plasmid map for pPEX10-1. FIG. **7**B diagrams the development of *Yarrowia lipolytica* strain Y4184U.

[0030] FIG. **8** provides plasmid maps for the following: (A) pZKL1-2SP98C; and, (B) pZKL2-5U89GC.

[0031] FIG. **9** provides plasmid maps for the following: (A) pYPS161; and, (B) pYRH13.

[0032] FIG. **10** diagrams the development of *Yarrowia lipolytica* strain Y4305U3.

[0033] FIG. **11** provides plasmid maps for the following: (A) pZKUM; and, (B) pZKD2-5U89A2.

[0034] FIG. **12** provides plasmid maps for the following: (A) pY87; and, (B) pY157.

[0035] The invention can be more fully understood from the following detailed description and the accompanying sequence descriptions, which form a part of this application.

[0036] The following sequences comply with 37 C.F.R. §1.821-1.825 ("Requirements for Patent Applications Containing Nucleotide Sequences and/or Amino Acid Sequence Disclosures—the Sequence Rules") and are consistent with World Intellectual Property Organization (WIPO) Standard ST.25 (1998) and the sequence listing requirements of the EPO and PCT (Rules 5.2 and 49.5(a-bis), and Section 208 and Annex C of the Administrative Instructions). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

[0037] SEQ ID NOs:1-86 are primers, ORFs encoding genes or proteins (or portions thereof, or plasmids, as identified in Table 1.

TABLE 1

Summary Of Nucleic Acid And Protein SEQ ID Numbers

Description and Abbreviation	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
Yarrowia lipolytica Pex1p (GenBank Accession No. CAG82178)	—	1 (1024 AA)
Yarrowia lipolytica Pex2p (GenBank Accession No. CAG77647)	—	2 (381 AA)
Yarrowia lipolytica Pex3p (GenBank Accession No. CAG78565)	—	3 (431 AA)
Yarrowia lipolytica Pex3Bp (GenBank Accession No. CAG83356)	—	4 (395 AA)
Yarrowia lipolytica Pex4p (GenBank Accession No. CAG79130)	—	5 (153 AA)
Yarrowia lipolytica Pex5p (GenBank Accession No. CAG78803)	—	6 (598 AA)
Yarrowia lipolytica Pex6p (GenBank Accession No. CAG82306)	—	7 (1024 AA)
Yarrowia lipolytica Pex7p (GenBank Accession No. CAG78389)	—	8 (356 AA)
Yarrowia lipolytica Pex8p (GenBank Accession No. CAG80447)	—	9 (671 AA)
Yarrowia lipolytica Pex10p (GenBank Accession No. CAG81606)	—	10 (377 AA)
Yarrowia lipolytica Pex12p (GenBank Accession No. CAG81532)	—	11 (408 AA)
Yarrowia lipolytica Pex13p (GenBank Accession No. CAG81789)	—	12 (412 AA)
Yarrowia lipolytica Pex14p (GenBank Accession No. CAG79323)	—	13 (380 AA)
Yarrowia lipolytica Pex16p (GenBank Accession No. CAG79622)	—	14 (391 AA)
Yarrowia lipolytica Pex17p (GenBank Accession No. CAG84025)	—	15 (225 AA)
Yarrowia lipolytica Pex19p (GenBank Accession No. AAK84827)	—	16 (324 AA)
Yarrowia lipolytica Pex20p (GenBank Accession No. CAG79226)	—	17 (417 AA)
Yarrowia lipolytica Pex22p (GenBank Accession No. CAG77876)	—	18 (195 AA)
Yarrowia lipolytica Pex26p (GenBank Accession No. NC_006072, antisense translation of nucleotides 117230-118387)	—	19 (386 AA)
Contig comprising *Yarrowia lipolytica* Pex10 gene encoding peroxisomal biogenesis factor protein (Pex10p) (GenBank Accession No. AB036770)	20 (3387 bp)	—
Yarrowia lipolytica Pex10 (GenBank Accession No. AB036770, nucleotides 1038-2171) (the protein sequence is 100% identical to SEQ ID NO: 10)	21 (1134 bp)	22 (377 AA)
Yarrowia lipolytica Pex10 (GenBank Accession No. AJ012084, which corresponds to nucleotides 1107-2171 of GenBank Accession No. AB036770) (the first 23 amino acids are truncated with respect to the protein sequences of SEQ ID NOs: 10 and 22)	23 (1065 bp)	24 (354 AA)
Yarrowia lipolytica Pex10p C_3HC_4 zinc ring finger motif (i.e., amino acids 327-364 of SEQ ID NO: 10)	—	25 (38 AA)
Yarrowia lipolytica truncated Pex10p (GenBank Accession No. CAG81606 [SEQ ID NO: 10], with C-terminal 32 amino acid deletion)	—	26 (345 AA)
Yarrowia lipolytica mutant acetohydroxyacid synthase (AHAS) gene comprising a W497L mutation	27 (2987 bp)	—
Plasmid pZP3-Pa777U	28 (13,066 bp)	—
Plasmid pY117	29 (9570 bp)	—
Plasmid pZP2-2988	30 (15,743 bp)	—

TABLE 1-continued

Summary Of Nucleic Acid And Protein SEQ ID Numbers

Description and Abbreviation	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
Plasmid pZKUE3S	31 (6303 bp)	—
Primer pZP-GW-5-1	32	—
Primer pZP-GW-5-2	33	—
Primer pZP-GW-5-3	34	—
Primer pZP-GW-5-4	35	—
Primer pZP-GW-3-1	36	—
Primer pZP-GW-3-2	37	—
Primer pZP-GW-3-3	38	—
Primer pZP-GW-3-4	39	—
Genome Walker adaptor [top strand]	40	—
Genome Walker adaptor [bottom strand]	41	—
Nested adaptor primer	42	—
Primer Per10 F1	43	—
Primer ZPGW-5-5	44	—
Primer Per10 R	45	—
Plasmid pFBAIN-MOD-1	46 (7222 bp)	—
Plasmid pFBAIn-PEX10	47 (8133 bp)	—
Primer PEX10-R-BsiWI	48	—
Primer PEX10-F1-SalI	49	—
Primer PEX10-F2-SalI	50	—
Plasmid pEXP-MOD1	51 (7277 bp)	—
Plasmid pPEX10-1	52 (7559 bp)	—
Plasmid pPEX10-2	53 (8051 bp)	—
Plasmid pZKL1-2SP98C	54 (15,877 bp)	—
Plasmid pZKL2-5U89GC	55 (15,812 bp)	—
Plasmid pYPS161	56 (7966 bp)	—
Primer Pex-10del1 3'.Forward	57	—
Primer Pex-10del2 5'.Reverse	58	—
Plasmid pYRH13	59 (8673 bp)	—
Primer PEX16Fii	60	—
Primer PEX16Rii	61	—
Primer 3UTR-URA3	62	—
Primer Pex16-conf	63	—
Real time PCR primer ef-324F	64	—
Real time PCR primer ef-392R	65	—
Real time PCR primer Pex16-741F	66	—
Real time PCR primer Pex16-802R	67	—
Nucleotide portion of TaqMan probe ef-345T	68	—
Nucleotide portion of TaqMan probe PEX16-760T	69	—
Plasmid pZKUM	70 (4313 bp)	—
Plasmid pZKD2-5U89A2	71 (15,966 bp)	—
Yarrowia lipolytica diacylglycerol acyltransferase (DGAT2) (U.S. Pat. No. 7,267,976)	72 (2119 bp)	73 (514 AA)
Synthetic Δ12 desaturase derived from *Fusarium moniliforme*, codon-optimized for expression in *Yarrowia lipolytica* ("FmD12S")	74 (1434 bp)	75 (477 AA)
Synthetic mutant Δ8 desaturase ("EgD8M"), derived from Euglena gracilis ("EgD8S"; U.S. Pat. No. 7,256,033)	76 (1272 bp)	77 (422 AA)
Synthetic Δ9 elongase derived from *Eutreptiella* sp. CCMP389 codon-optimized for expression in *Yarrowia lipolytica* ("E389D9eS")	78 (792 bp)	79 (263 AA)
Synthetic Δ5 desaturase derived from *Euglena gracilis*, codon-optimized for expression in *Yarrowia lipolytica* ("EgD5S")	80 (1350 bp)	81 (449 AA)

TABLE 1-continued

Summary Of Nucleic Acid And Protein SEQ ID Numbers

Description and Abbreviation	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
Plasmid pY157	82 (6356 bp)	—
Plasmid pY87	83 (5910 bp)	—
Escherichia coli LoxP recombination site, recognized by a Cre recombinase enzyme	84 (34 bp)	—
Primer UP 768	85	—
Primer LP 769	86	—

DETAILED DESCRIPTION OF THE INVENTION

[0038] Described herein are generalized methods to manipulate the concentration (as a percent of total fatty acids) and content (as a percent of the dry cell weight) of long-chain polyunsaturated fatty acids ["LC-PUFAs"] in PUFA-producing eukaryotic organisms. These methods rely on disruption of a native peroxisome biogenesis factor ["Pex"] protein within the host and will have wide-spread applicability to a variety of eukaryotic organisms having native or genetically-engineered ability to produce PUFAs, including algae, fungi, oomycetes, yeast, euglenoids, stramenopiles, plants and some mammalian systems.

[0039] PUFAs, or derivatives thereof, are used as dietary substitutes, or supplements, particularly infant formulas, for patients undergoing intravenous feeding or for preventing or treating malnutrition. For example, PUFAs may be incorporated into cooking oils, fats or margarines and ingested as part of a consumer's typical diet, thereby giving the consumer desired dietary supplementation. Further, PUFAs may also be incorporated into infant formulas, nutritional supplements or other food products and may find use as anti-inflammatory or cholesterol lowering agents. Optionally, the compositions may be used for pharmaceutical use, either human or veterinary.

DEFINITIONS

[0040] In this disclosure, a number of terms and abbreviations are used.

[0041] The following definitions are provided.

[0042] "Open reading frame" is abbreviated as "ORF".

[0043] "Polymerase chain reaction" is abbreviated as "PCR".

[0044] "American Type Culture Collection" is abbreviated as "ATCC".

[0045] "Polyunsaturated fatty acid(s)" is abbreviated as "PUFA(s)".

[0046] "Triacylglycerols" are abbreviated as "TAGs".

[0047] "Total fatty acids" are abbreviated as "TFAs".

[0048] "Fatty acid methyl esters" are abbreviated as "FAMEs".

[0049] "Dry cell weight" is abbreviated as "DCW".

[0050] The term "invention" or "present invention" as used herein is not meant to be limiting but applies generally to any of the inventions defined in the claims or described herein.

[0051] The term "peroxisomes" refers to ubiquitous organelles found in all eukaryotic cells. They have a single lipid bilayer membrane that separates their contents from the cytosol and that contains various membrane proteins essential to the functions described below. Peroxisomes selectively import proteins via an "extended shuttle mechanism". More specifically, there are at least 32 known peroxisomal proteins, also known as peroxins, which participate in the process of importing proteins by means of ATP hydrolysis through the peroxisomal membrane. Some peroxins comprise a specific protein signal, i.e., a peroxisomal targeting signal or "PTS", at either the N-terminus or C-terminus to signal that importation through the peroxisomal membrane should occur. Once cellular proteins are imported into the peroxisome, they are typically subjected to some means of degradation. For example, peroxisomes contain oxidative enzymes, such as catalase, D-amino acid oxidase and uric acid oxidase, that enable degradation of substances that are toxic to the cell. Alternatively, peroxisomes breakdown fatty acid molecules to produce free molecules of acetyl-CoA which are exported back to the cytosol, in a process called β-oxidation.

[0052] The terms "peroxisome biogenesis factor protein", "peroxin" and "Pex protein" are interchangeable and refer to proteins involved in peroxisome biogenesis and/or that participate in the process of importing cellular proteins by means of ATP hydrolysis through the peroxisomal membrane. The acronym of a gene that encodes any of these proteins is "Pex gene". A system for nomenclature of Pex genes is described by Distel et al., *J. Cell Biol.,* 135:1-3 (1996). At least 32 different Pex genes have been identified so far in various eukaryotic organisms. Many Pex genes have been isolated from the analysis of mutants that demonstrated abnormal peroxisomal functions or structures. Based on a review by Kiel, J. A. K. W., et al. (*Traffic,* 7:1291-1303 (2006)), wherein in silico analysis of the genomic sequences of 17 different fungal species was performed, the following Pex proteins were identified: Pex1p, Pex2p, Pex3p, Pex3Bp, Pex4p, Pex5p, Pex5Bp, Pex5Cp, Pex5/20p, Pex6p, Pex7p, Pex8p, Pex10p, Pex12p, Pex13p, Pex14p, Pex15p, Pex16p, Pex17p, Pex14/17p, Pex18p, Pex19p, Pex20p, Pex21p, Pex21Bp, Pex22p, Pex22p-like and Pex26p. Thus, each of these proteins is referred to herein as a "Pex protein", a "peroxin" or a "peroxisome biogenesis factor protein", and is encoded by at least one "Pex gene".

[0053] The term "conserved domain" or "motif" refers to 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 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. Of relevance herein, Pex2p, Pex10p and Pex12p all share a cysteine-rich motif near their carboxyl termini, known as a C_3HC_4 zinc ring finger motif. This motif appears to be required for their activities, involved in protein docking and translocation into the peroxisome (Kiel, J. A. K. W., et al., *Traffic,* 7:1291-1303 (2006)).

[0054] The term "C_3HC_4 zinc ring finger motif" or "C_3HC_4 motif" generically refers to a conserved cysteine-rich motif that binds two zinc ions, identified by the presence of a sequence of amino acids as set forth in Formula I:

$$CX_2CX_{9-27}CX_{1-3}HX_2CX_2CX_{4-48}CX_2C \qquad \text{Formula I}$$

The C_3HC_4 zinc ring finger motif within the *Yarrowia lipolytica* gene encoding the peroxisome biogenesis factor 10 protein, i.e., YIPex10p, is located between amino acids 327-364 of SEQ ID NO:10 and is defined by a $CX_2CX_{11}CX_1HX_2CX_2CX_{10}CX_2C$ motif (SEQ ID NO:25). The C_3HC_4 zinc ring finger motif within the *Y. lipolytica* gene

encoding the peroxisome biogenesis factor 2 protein, i.e., YlPex2p, is located between amino acids 266-323 of SEQ ID NO:2. The *Y. lipolytica* peroxisome biogenesis factor 12 protein, i.e., YlPex12p, contains an imperfect C_3HC_4 ring-finger motif located between amino acids 342-391 of SEQ ID NO:11. The protein sequences corresponding to the C_3HC_4 zinc ring finger motif of YlPex10, YlPex2 and YlPex12 are aligned in FIG. **2**A; asterisks denote the conserved cysteine or histidine residues of the motif.

[0055] YlPex10, YlPex2 and YlPex12 are thought to form a ring finger complex by protein-protein interaction. The proposed interaction between the cystine and histidine residues of the YlPex10p C_3HC_4 finger motif with two zinc residues is schematically diagrammed in FIG. **2**B.

[0056] The term "Pex10" refers to the gene encoding the peroxisome biogenesis factor 10 protein or peroxisomal assembly protein Peroxin 10, wherein the peroxin protein is hereinafter referred to as "Pex10p". The function of Pex10p has not been clearly elucidated, although studies in other organisms have revealed that Pex10 products are localized in the peroxisomal membrane and are essential to the normal functioning of the organelle. A C_3HC_4 zinc ring finger motif appears to be conserved in the C-terminal region of Pex10p (Kalish, J. E. et al., *Mol. Cell. Biol.,* 15:6406-6419 (1995); Tan, X. et al., *J. Cell Biol.,* 128:307-319 (1995); Warren, D. S., et al., *Am. J. Hum. Genet.,* 63:347-359 (1998)) and is required for enzymatic activity.

[0057] The term "YlPex10" refers to the *Yarrowia lipolytica* gene encoding the peroxisome biogenesis factor 10 protein, wherein the protein is hereinafter referred to as "YlPex10p". This particular peroxin was recently studied by Sumita et al. (*FEMS Microbiol. Lett.,* 214:31-38 (2002)). The nucleotide sequence of YlPex10 was registered in GenBank under multiple accession numbers, including GenBank Accession No. CAG81606 (SEQ ID NO:10), No. AB036770 (SEQ ID NOs:20, 21 and 22) and No. AJ012084 (SEQ ID NOs:23 and 24). The YlPex10p sequence set forth in SEQ ID NO:24 is 354 amino acids in length. In contrast, the YlPex10p sequences set forth in SEQ ID NO:10 and SEQ ID NO:22 are each 377 amino acids in length, as the 100% identical sequences possess an additional 23 amino acids at the N-terminus of the protein (corresponding to a different start codon than that identified in GenBank Accession No. AJ012084 (SEQ ID NO:24)).

[0058] The term "Pex3" refers to the gene encoding the peroxisome biogenesis factor 3 protein or peroxisomal assembly protein Peroxin 3, wherein the peroxin protein is hereinafter referred to as "Pex3p". Although mechanistic details concerning the function of Pex3p have not been clearly resolved, it is clear that Pex3p is a peroxisomal integral membrane protein required early in peroxisome biogenesis for formation of the peroxisomal membrane (see, e.g., Baerends, R. J. et al., *J. Biol. Chem.,* 271:8887-8894 (1996); Bascom, R. A. et al, *Mol. Biol. Cell,* 14:939-957 (2003)).

[0059] The term "YlPex3" refers to the *Yarrowia lipolytica* gene encoding the peroxisome biogenesis factor 3 protein, wherein the protein is hereinafter referred to as "YlPex3p". The nucleotide sequence of YlPex3 was registered in GenBank as Accession No. CAG78565 (SEQ ID NO:3).

[0060] The term "Pex16" refers to the gene encoding the peroxisome biogenesis factor 16 protein or peroxisomal assembly protein Peroxin 16, wherein the peroxin protein is hereinafter referred to as "Pex16p". The function of Pex16p has not been clearly elucidated, although studies in various organisms have revealed that Pex16 products play a role in the formation of the peroxisomal membrane and regulation of peroxisomal proliferation (Platta, H. W. and R. Erdmann, *Trends Cell Biol.,* 17(10):474-484 (2007)).

[0061] The term "YlPex16" refers to the *Yarrowia lipolytica* gene encoding the peroxisome biogenesis factor 16 protein, wherein the protein is hereinafter referred to as "YlPex16p". This particular peroxin was described by Elizen G. A., et al. (*J. Cell Biol.,* 137:1265-1278 (1997)) and Titorenko, V. I. et al. (*Mol. Cell. Biol.,* 17:5210-5226 (1997)). The nucleotide sequence of YlPex16 was registered in GenBank as Accession No. CAG79622 (SEQ ID NO:14).

[0062] The term "disruption" in or in connection with a native Pex gene refers to an insertion, deletion, or targeted mutation within a portion of that gene, that results in either a complete gene knockout such that the gene is deleted from the genome and no protein is translated or a translated Pex protein having an insertion, deletion, amino acid substitution or other targeted mutation. The location of the disruption in the protein may be, for example, within the N-terminal portion of the protein or within the C-terminal portion of the protein. The disrupted Pex protein will have impaired activity with respect to the Pex protein that was not disrupted, and can be non-functional. A disruption in a native gene encoding a Pex protein also includes alternate means that result in low or lack of expression of the Pex protein, such as could result via manipulating the regulatory sequences, transcription and translation factors and/or signal transduction pathways or by use of sense, antisense or RNAi technology, etc.

[0063] As used herein, the term "Pex-disrupted organism" refers to any oleaginous eukaryotic organism comprising genes that encode a functional polyunsaturated fatty acid biosynthetic pathway and having a disruption, as defined above, in a native gene that encodes a peroxisome biogenesis factor protein,

[0064] The term "lipids" refer to any fat-soluble (i.e., lipophilic), naturally-occurring molecule. Lipids are a diverse group of compounds that have many key biological functions, such as structural components of cell membranes, energy storage sources and intermediates in signaling pathways. Lipids may be broadly defined as hydrophobic or amphiphilic small molecules that originate entirely or in part from either ketoacyl or isoprene groups. A general overview of lipids, based on the Lipid Metabolites and Pathways Strategy (LIPID MAPS) classification system (National Institute of General Medical Sciences, Bethesda, Md.), is shown below in Table 2.

Table 2

Overview of Lipid Classes

[0065]

Structural Building Block	Lipid Category	Examples Of Lipid Classes
Derived from condensation of ketoacyl subunits	Fatty Acyls	Includes fatty acids, eicosanoids, fatty esters and fatty amides
	Glycerolipids	Includes mainly of mono-, di- and tri-substituted glycerols, the most well-known being the fatty acid esters of glycerol ["triacylglycerols"]

-continued

Structural Building Block	Lipid Category	Examples Of Lipid Classes
	Glycero-phospholipids or Phospholipids	Includes phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositols and phosphatidic acids
	Sphingolipids	Includes ceramides, phospho-sphingolipids (e.g., sphingomyelins), glycosphingolipids (e.g., gangliosides), sphingosine, cerebrosides
	Saccharolipids	Includes acylaminosugars, acylamino-sugar glycans, acyltrehaloses, acyltrehalose glycans
	Polyketides	Includes halogenated acetogenins, polyenes, linear tetracyclines, polyether antibiotics, flavonoids, aromatic polyketides
Derived from condensation of isoprene subunits	Sterol Lipids	Includes sterols (e.g., cholesterol), C18 steroids (e.g., estrogens), C19 steroids (e.g., androgens), C21 steroids (e.g., progestogens, glucocorticoids and mineral-ocorticoids), secosteroids, bile acids
	Prenol Lipids	Includes isoprenoids, carotenoids, quinones, hydroquinones, polyprenols, hopanoids

[0066] The term "total lipid fraction" of cells herein refers to all esterified fatty acids of the cell. Various subfractions within the total lipid fraction can be isolated, including the triacylglycerol ["oil"] fraction, phosphatidylcholine fraction and the phosphatidylethanolamine fraction, although this is by no means inclusive of all sub-fractions.

[0067] "Lipid bodies" refer to lipid droplets that are bound by a monolayer of phospholipid and, usually, by specific proteins. These organelles are sites where most organisms transport/store neutral lipids. Lipid bodies are thought to arise from microdomains of the endoplasmic reticulum that contain TAG biosynthesis enzymes. Their synthesis and size appear to be controlled by specific protein components.

[0068] "Neutral lipids" refer to those lipids commonly found in cells in lipid bodies as storage fats and oils 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.

[0069] The terms "triacylglycerols" ["TAGs"] and "oil" are interchangeable and refer to neutral lipids composed of three fatty acyl residues esterified to a glycerol molecule. TAGs can contain long chain PUFAs, as well as shorter saturated and unsaturated fatty acids and longer chain saturated fatty acids. The TAG fraction of cells is also referred to as the "oil fraction", and "oil biosynthesis" generically refers to the synthesis of TAGs in the cell. The oil or TAG fraction is a sub-fraction of the total lipid fraction, although also it constitutes a major part of the total lipid content, measured as the weight of total fatty acids in the cell as a percent of the dry cell weight [see below], in oleaginous organisms. The fatty acid composition in the oil ["TAG"] fraction and the fatty acid composition of the total lipid fraction are generally similar. Thus, an increase or decrease in the concentration of PUFAs in the total lipid fraction will correspond with an increase or decrease in the concentration of PUFAs in the oil ["TAG"] fraction, and vice versa.

[0070] The term "total fatty acids" ["TFAs"] herein refer 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 total lipid fraction or the oil fraction, for example. Thus, total fatty acids include fatty acids from neutral and polar lipid fractions, including the phosphatidylcholine fraction, the phosphatidylethanolamine fraction and the diacylglycerol, monoacylglycerol and triacylglycerol ["TAG or oil"] fractions but not free fatty acids.

[0071] The term "total lipid content" of cells is a measure of TFAs as a percent of the dry cell weight ["DCW"]. Thus, total lipid content ["TFAs % DCW"] is equivalent to, e.g., milligrams of total fatty acids per 100 milligrams of DCW.

[0072] Generally, the concentration of a fatty acid 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 in the disclosure 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).

[0073] In some cases, it is useful to express the content of a given fatty acid(s) in a cell as its percent of the dry cell weight ["% DCW"]. Thus, for example, eicosapentaenoic acid % DCW would be determined according to the following formula: (eicosapentaenoic acid % TFAs)*(TFA % DCW)]/100.

[0074] The terms "lipid profile" and "lipid composition" are interchangeable and refer to the amount of an individual fatty acid contained in a particular lipid fraction, such as in the total lipid fraction or the oil ["TAG"] fraction, wherein the amount is expressed as a percent of TFAs. The sum of each individual fatty acid present in the mixture should be 100.

[0075] As used herein, the term "fold increase" refers to an increase obtained by multiplying by a number. For example, multiplying by 1.3 a quantity, an amount, a concentration, a weight percent, etc. provides a 1.3 fold increase.

[0076] 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" ["ω-6" or "n-6"] versus "omega-3 fatty acids" ["ω-3" or "n-3"] are provided in U.S. Pat. No. 7,238,482, which is hereby incorporated herein by reference.

[0077] 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 the Table summarizes the common names of ω-3 and ω-6 fatty acids and their precursors, the abbreviations that are 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 ω-6
γ-Linolenic	GLA	cis-6,9,12-octadecatrienoic	18:3 ω-6
Eicosadienoic	EDA	cis-11,14-eicosadienoic	20:2 ω-6
Dihomo-γ-Linolenic	DGLA	cis-8,11,14-eicosatrienoic	20:3 ω-6
Arachidonic	ARA	cis-5,8,11,14-eicosatetraenoic	20:4 ω-6
α-Linolenic	ALA	cis-9,12,15-octadecatrienoic	18:3 ω-3
Stearidonic	STA	cis-6,9,12,15-octadecatetraenoic	18:4 ω-3
Eicosatrienoic	ETrA	cis-11,14,17-eicosatrienoic	20:3 ω-3
Sciadonic	SCI	cis-5,11,14-eicosatrienoic	20:3b ω-6
Juniperonic	JUP	cis-5,11,14,17-eicosatetraenoic	20:4b ω-3
Eicosa-tetraenoic	ETA	cis-8,11,14,17-eicosatetraenoic	20:4 ω-3
Eicosa-pentaenoic	EPA	cis-5,8,11,14,17-eicosapentaenoic	20:5 ω-3
Docosatrienoic	DRA	cis-10,13,16-docosatrienoic	22:3 ω-3
Docosa-tetraenoic	DTA	cis-7,10,13,16-docosatetraenoic	22:4 ω-3
Docosa-pentaenoic	DPAn-6	cis-4,7,10,13,16-docosapentaenoic	22:5 ω-6
Docosa-pentaenoic	DPA	cis-7,10,13,16,19-docosapentaenoic	22:5 ω-3
Docosa-hexaenoic	DHA	cis-4,7,10,13,16,19-docosahexaenoic	22:6 ω-3

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

[0078] As used herein, the terms "a combination of polyunsaturated fatty acids" or "any combination of polyunsaturated fatty acids" refers to a mixture of any two or more of the polyunsaturated fatty acids listed above in Table 3. Such combination has the attributes of a concentration and of a weight percent that can be measured relative to a variety of concentrations or weight percents in the cell, including relative to the weight percent of the total fatty acids in the cell.

[0079] A metabolic pathway, or biosynthetic pathway, in a biochemical sense, can be regarded as a series of chemical reactions occurring in order within a cell, catalyzed by enzymes, to achieve either the formation of a metabolic product to be used or stored by the cell, or the initiation of another metabolic pathway, which is termed "flux generating step". Many of these pathways are elaborate, and involve a step by step modification of the initial substance to shape it into a product having the exact chemical structure desired.

[0080] The term "PUFA biosynthetic pathway" refers to a metabolic process that converts oleic acid to ω-6 fatty acids such as LA, EDA, GLA, DGLA, ARA, DRA, DTA and DPAn-6 and ω-3 fatty acids such as ALA, STA, ETrA, ETA, EPA, DPA and DHA. This process is well described in the literature. See e.g., Int'. App. Pub. No. WO 2006/052870. Briefly, this process involves elongation of the carbon chain through the addition of carbon atoms and desaturation of the elongated 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 them) associated with the biosynthesis of a PUFA, including: a Δ4 desaturase, a Δ5 desaturase, a Δ6 desaturase, a Δ12 desaturase, a Δ15 desaturase, a Δ17 desaturase, a Δ9 desaturase, a Δ8 desaturase, a Δ9 elongase, a $C_{14/16}$ elongase, a $C_{16/18}$ elongase, a $C_{18/20}$ elongase and/or a $C_{20/22}$ elongase.

[0081] The term "ω-3/ω-6 fatty acid biosynthetic pathway" refers to a set of genes which, when expressed under the appropriate conditions, encode enzymes that catalyze the production of either or both ω-3 and ω-6 fatty acids. Typically the genes involved in the ω-3/ω-6 fatty acid biosynthetic pathway encode PUFA biosynthetic pathway enzymes. A representative pathway is illustrated in FIG. **1**, providing for the conversion of myristic acid through various intermediates to DHA, which demonstrates how both ω-3 and ω-6 fatty acids may be produced from a common source. The pathway is naturally divided into two portions, such that one portion generates only ω-3 fatty acids and the other portion, only ω-6 fatty acids. That portion that generates only ω-3 fatty acids is referred to herein as the ω-3 fatty acid biosynthetic pathway, whereas that portion that generates only ω-6 fatty acids is referred to herein as the ω-6 fatty acid biosynthetic pathway.

[0082] The term "functional" as used herein relating to the ω-3/ω-6 fatty acid biosynthetic pathway, means that some (or all) of the genes in the pathway express active enzymes, resulting in in vivo catalysis or substrate conversion. It should be understood that "ω-3/ω-6 fatty acid biosynthetic pathway" or "functional ω-3/ω-6 fatty acid biosynthetic pathway" does not imply that all of the genes listed in the above paragraph are required, as a number of fatty acid products require only the expression of a subset of the genes of this pathway.

[0083] The term "Δ6 desaturase/Δ6 elongase pathway" refers to a PUFA biosynthetic pathway that minimally includes at least one Δ6 desaturase and at least one $C_{16/20}$ elongase, thereby enabling biosynthesis of DGLA and/or ETA from LA and ALA, respectively, with GLA and/or STA as intermediate fatty acids. With expression of other desaturases and elongases, ARA, EPA, DPA and DHA may also be synthesized.

[0084] The term "Δ9 elongase/Δ8 desaturase pathway" refers to a PUFA biosynthetic pathway that minimally includes at least one Δ9 elongase and at least one Δ8 desaturase, thereby enabling biosynthesis of DGLA and/or ETA from LA and ALA, respectively, with EDA and/or ETrA as intermediate fatty acids. With expression of other desaturases and elongases, ARA, EPA, DPA and DHA may also be synthesized.

[0085] The term "desaturase" refers to a polypeptide that can desaturate adjoining carbons in a fatty acid by removing a hydrogen from one of the adjoining carbons and thereby introducing a double bond between them. Desaturation produces a fatty acid or precursor of interest. Despite use of the omega-reference system throughout the specification to refer to specific fatty acids, it is more convenient to indicate the activity of a desaturase by counting from the carboxyl end of the substrate using the delta-system. Of particular interest

herein are: 1) Δ5 desaturases that catalyze the conversion of the substrate fatty acid, DGLA, to ARA and/or of the substrate fatty acid, ETA, to EPA; 2) Δ17 desaturases that desaturate a fatty acid between the 17^{th} and 18^{th} carbon atom numbered from the carboxyl-terminal end of the molecule and which, for example, catalyze the conversion of the substrate fatty acid, ARA, to EPA and/or the conversion of the substrate fatty acid, DGLA, to ETA; 3) Δ6 desaturases that catalyze the conversion of the substrate fatty acid, LA, to GLA and/or the conversion of the substrate fatty acid, ALA, to STA; 4) Δ12 desaturases that catalyze the conversion of the substrate fatty acid, oleic acid, to LA; 5) Δ15 desaturases that catalyze the conversion of the substrate fatty acid, LA, to ALA and/or the conversion of the substrate fatty acid, GLA, to STA; 6) Δ4 desaturases that catalyze the conversion of the substrate fatty acid, DPA, to DHA and/or the conversion of the substrate fatty acid, DTA, to DPAn-6; 7) Δ8 desaturases that catalyze the conversion of the substrate fatty acid, EDA, to DGLA and/or the conversion of the substrate fatty acid, ETrA, to ETA; and, 8) Δ9 desaturases that catalyze the conversion of the substrate fatty acid, palmitate, to palmitoleic acid (16:1) and/or the conversion of the substrate fatty acid, stearic acid, to oleic acid. Δ15 and Δ17 desaturases are also occasionally referred to as "omega-3 desaturases", "w-3 desaturases", and/or "ω-3 desaturases", based on their ability to convert ω-6 fatty acids into their ω-3 counterparts (e.g., conversion of LA into ALA and ARA into EPA, respectively). It may be desirable to empirically determine the specificity of a particular fatty acid desaturase by transforming a suitable host with the gene for the fatty acid desaturase and determining its effect on the fatty acid profile of the host.

[0086] The term "elongase" refers to a polypeptide that can elongate a fatty acid carbon chain to produce an acid 2 carbons longer than the fatty acid substrate that the elongase acts upon. This process of elongation occurs in a multi-step mechanism in association with fatty acid synthase, as described in U.S. Pat. App. Pub. No. 2005/0132442 and Int'l App. Pub. No. WO 2005/047480. Examples of reactions catalyzed by elongase systems are the conversion of GLA to DGLA, STA to ETA and EPA to DPA. In general, the substrate selectivity of elongases is somewhat broad but segregated by both chain length and the degree and type of unsaturation. For example, a $C_{14/16}$ elongase utilizes a C_{14} substrate e.g., myristic acid, a $C_{16/18}$ elongase utilizes a C_{16} substrate e.g., palmitate, a $C_{18/20}$ elongase [also known as a Δ6 elongase as the terms can be used interchangeably] utilizes a C_{18} substrate e.g., GLA or STA, and a $C_{20/22}$ elongase utilizes a C_{20} substrate e.g., EPA. In like manner, a Δ9 elongase is able to catalyze the conversion of LA and ALA to EDA and ETrA, respectively. It is important to note that some elongases have broad specificity and thus a single enzyme may be capable of catalyzing several elongase reactions. For example a single enzyme may thus act as both a $C_{16/18}$ elongase and a $C_{18/20}$ elongase.

[0087] The terms "conversion efficiency" and "percent substrate conversion" refer to the efficiency by which a particular enzyme, such as a desaturase, 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 in the pathway derived from it.

[0088] The term "oleaginous" refers to those organisms that tend to store their energy source in the form of oil (Weete, In: Fungal Lipid Biochemistry, 2^{nd} Ed., Plenum, 1980).

[0089] The term "oleaginous yeast" refers to those microorganisms classified as yeasts that can make oil, that is, TAGs. Generally, the cellular oil or TAG content of oleaginous microorganisms follows a sigmoid curve, wherein the concentration of lipid increases until it reaches a maximum at the late logarithmic or early stationary growth phase and then gradually decreases during the late stationary and death phases (Yongmanitchai and Ward, *Appl. Environ. Microbiol.*, 57:419-25 (1991)). Oleaginous microorganisms as referred to herein typically accumulate in excess of about 25% of their dry cell weight as oil or TAGs. Examples of oleaginous yeast include, but are not limited to, the following genera: *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon and Lipomyces.*

[0090] As used herein, the terms "isolated nucleic acid fragment" and "isolated nucleic acid molecule" are used interchangeably and refer to 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.

[0091] 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,* 2^{nd} ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y. (1989), which is hereby incorporated herein by reference, particularly Chapter 11 and Table 11.1.

[0092] 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 of thirty or more contiguous nucleotides is necessary in order to putatively identify 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 microbial 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.

[0093] 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.

[0094] The terms "homology" and "homologous" are used interchangeably herein. They refer to nucleic acid fragments

wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the Pex nucleic acid fragments described herein, such as 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.

[0095] Moreover, the skilled artisan recognizes that homologous nucleic acid sequences are also defined by their ability to hybridize, under moderately stringent conditions, such as 0.5×SSC, 0.1% SDS, 60° C., with the sequences exemplified herein, or to any portion of the nucleotide sequences disclosed herein and which are functionally equivalent thereto.

[0096] "Codon degeneracy" refers to the nature in the genetic code permitting variation of the nucleotide sequence without effecting 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.

[0097] "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.

[0098] "Gene" refers to a nucleic acid fragment that expresses a specific protein, and which may refer to the coding region alone or may include regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "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. 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.

[0099] "Coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, enhancers, silencers, 5' untranslated leader sequence (e.g., between the transcription start site and the translation initiation codon), introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures.

[0100] "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 have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

[0101] The terms "3' non-coding sequences" and "transcription terminator" refer to DNA sequences located 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.

[0102] "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. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Pat. No. 5,107,065; Int'l. App. Pub. No. WO 99/28508). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated and yet has an effect on cellular processes.

[0103] 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 con-

trol of the promoter. Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

[0104] The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from nucleic acid fragments. Expression may also refer to translation of mRNA into a polypeptide.

[0105] "Mature" protein refers to a post-translationally processed polypeptide, i.e., one from which any pre- or pro-peptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA, i.e., with pre- and pro-peptides still present. Pre- and pro-peptides may be, but are not limited to, intracellular localization signals.

[0106] "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" organisms.

[0107] "Stable transformation" refers to the transfer of a nucleic acid fragment into a genome of a host organism, including both nuclear and organellar genomes, resulting in genetically stable inheritance. In contrast, "transient transformation" refers to the transfer of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without integration or stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms.

[0108] The terms "plasmid" and "vector" refer to an extra chromosomal 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 be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, 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.

[0109] The term "expression cassette" refers to a fragment of DNA comprising 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., open reading frame ["ORF"] and, 3) a 3' untranslated region, i.e., a terminator that in eukaryotes usually contains a polyadenylation site. 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.

[0110] The term "percent identity" refers to a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. "Identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the percentage of match between compared sequences. "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.) Humania: 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).

[0111] Preferred methods to determine percent identity are designed to give the best match between the sequences tested. Methods to determine percent identity and percent similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). 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™ v6.1 program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.). 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.

[0112] It is well understood by one skilled in the art that various measures of sequence percent identity are useful in identifying polypeptides, from other species, wherein such polypeptides have the same or similar function or activity. Useful examples of percent identities include, but are not limited to, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or any integer percentage from 50% to 100%. Indeed, any integer amino acid identity from 50% to 100% may be useful in describing suitable nucleic acid fragments (isolated polynucleotides) encoding polypeptides in methods and host cells described herein, such as 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%. In some cases, suitable nucleic acid fragments (isolated polynucleotides) encode polypeptides that are at least about 70% identical, preferably at least about 75% identical, and more preferably at least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are at least about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein.

[0113] 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.

[0114] The term "sequence analysis software" refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software include, but is not limited to: 1) the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis.); 2) BLASTP, BLASTN, BLASTX (Altschul et al., *J. Mol. Biol.*, 215:403-410 (1990)); 3) DNASTAR (DNASTAR, Inc. Madison, Wis.); 4) Sequencher (Gene Codes Corporation, Ann Arbor, Mich.); and, 5) the FASTA program incorporating the Smith-Waterman algorithm (W. R. Pearson, *Comput. Methods Genome Res.*, [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Plenum: New York, N.Y.). Within this description, whenever sequence analysis software is used for analysis, the analytical results are based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" means any set of values or parameters that originally load with the software when first initialized.

[0115] Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual,* 2^{nd} ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y. (1989) (hereinafter "Maniatis"); by Silhavy, T. J., Bennan, M. L. and Enquist, L. W., *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y. (1984); and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, published by Greene Publishing Assoc. and Wiley-Interscience, Hoboken, N.J. (1987).

An Overview Biosynthesis of Fatty Acids and Triacylglycerols

[0116] In general, lipid accumulation in oleaginous microorganisms is triggered in response to the overall carbon to nitrogen ratio present in the growth medium. This process, leading to the de novo synthesis of free palmitate (16:0) in oleaginous microorganisms, is described in detail in U.S. Pat. No. 7,238,482. Palmitate is the precursor of longer-chain saturated and unsaturated fatty acid derivates, which are formed through the action of elongases and desaturases (FIG. **1**).

[0117] TAGs, the primary storage unit for fatty acids, are formed by a series of reactions that involve: 1) esterification of one molecule of acyl-CoA to glycerol-3-phosphate via an acyltransferase to produce lysophosphatidic acid; 2) esterification of a second molecule of acyl-CoA via an acyltransferase to yield 1,2-diacylglycerol phosphate, commonly identified as phosphatidic acid; 3) removal of a phosphate by phosphatidic acid phosphatase to yield 1,2-diacylglycerol ["DAG"]; and, 4) addition of a third fatty acid by the action of an acyltransferase to form the TAG.

[0118] A wide spectrum of fatty acids can be incorporated into TAGs, including saturated and unsaturated fatty acids and short-chain and long-chain fatty acids. Some non-limiting examples of fatty acids that can be incorporated into TAGs by acyltransferases include: capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), vaccenic (18:1), LA (18:2), eleostearic (18:3), GLA (18:3), ALA (18:3), STA (18:4), arachidic (20:0), EDA (20:2), DGLA (20:3), ETrA (20:3), ARA (20:4), ETA (20:4), EPA (20:5), behenic (22:0), DPA (22:5), DHA (22:6), lignoceric (24:0), nervonic (24:1), cerotic (26:0) and montanic (28:0) fatty acids. In the methods and host cells described herein, incorporation of "long-chain" PUFAs into TAGs may be most desirable, wherein long-chain PUFAs include any fatty acid derived from an 18:1 substrate having at least 18 carbons in length, i.e., C_{18} or greater. This also includes hydroxylated fatty acids, epoxy fatty acids and conjugated linoleic acid.

[0119] Although most PUFAs are incorporated into TAGs as neutral lipids and are stored in lipid bodies, it is important to note that a measurement of the total PUFAs within an oleaginous organism should include those PUFAs that are located in the phosphatidylcholine fraction, phosphatidylethanolamine fraction, and triacylglycerol, also known as the TAG or oil, fraction.

Biosynthesis of Omega Fatty Acids

[0120] The metabolic process wherein oleic acid is converted to ω-3/ω6 fatty acids 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. **1** and as described below, there are often multiple alternate pathways for production of a specific ω-3/ω-6 fatty acid.

[0121] Specifically, FIG. **1** depicts the pathways described below. All pathways require the initial conversion of oleic acid to linoleic acid ["LA"], the first of the ω-6 fatty acids, by a Δ12 desaturase. Then, using the "Δ6 desaturase/Δ6 elongase pathway" and LA as substrate, long-chain ω-6 fatty acids are formed as follows: 1) LA is converted to γ-linolenic acid ["GLA"] by a Δ6 desaturase; 2) GLA is converted to dihomo-γ-linolenic acid ["DGLA"] by a $C_{18/20}$ elongase; 3) DGLA is converted to arachidonic acid ["ARA"] by a Δ5 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 Δ4 desaturase.

[0122] Alternatively, the "Δ6 desaturase/Δ6 elongase pathway" can use α-linolenic acid ["ALA"] as substrate to produce long-chain ω-3 fatty acids as follows: 1) LA is converted to ALA, the first of the ω-3 fatty acids, by a Δ15 desaturase; 2) ALA is converted to stearidonic acid ["STA"] by a Δ6 desaturase; 3) STA is converted to eicosatetraenoic acid ["ETA"] by a $C_{18/20}$ elongase; 4) ETA is converted to eicosapentaenoic acid ["EPA"] by a Δ5 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 Δ4 desaturase. Optionally, ω-6 fatty acids may be converted to ω-3 fatty acids. For example, ETA and EPA are produced from DGLA and ARA, respectively, by Δ17 desaturase activity.

[0123] Alternate pathways for the biosynthesis of ω-3/ω-6 fatty acids utilize Δ9 elongase and Δ8 desaturase, that is, the "Δ9 elongase/Δ8 desaturase pathway". More specifically, LA and ALA may be converted to EDA and ETrA, respectively, by a Δ9 elongase. A Δ8 desaturase then converts EDA to DGLA and/or ETrA to ETA. Downstream PUFAs are subsequently formed as described above.

[0124] The host organism herein must possess the ability to produce PUFAs, either naturally or via techniques of genetic engineering. Although many microorganisms can synthesize PUFAs (including ω-3/ω-6 fatty acids) in the ordinary course of cellular metabolism, some of whom could be commercially cultured, few to none of these organisms produce oils

having a desired oil content and composition for use in pharmaceuticals, dietary substitutes, medical foods, nutritional supplements, other food products, industrial oleochemicals or other end-use applications. Thus, there is increasing emphasis on the ability to engineer microorganisms for production of "designer" lipids and oils, wherein the fatty acid content and composition are carefully specified by genetic engineering. On this basis, it is expected that the host likely comprises heterologous genes encoding a functional PUFA biosynthetic pathway but not necessarily.

[0125] If the host organism does not natively produce the desired PUFAs or possess the desired lipid profile, one skilled in the art is familiar with the considerations and techniques necessary to introduce one or more expression cassettes encoding appropriate enzymes for PUFA biosynthesis into the host organism of choice. Numerous teachings are provided in the literature to one of skill for so introducing such expression cassettes into various host organisms. Some references using the host organism *Yarrowia lipolytica* are provided as follows: U.S. Pat. No. 7,238,482; Int'l. App. Pub. No. WO 2006/033723, Pat. Appl. Pub. No. US-2006-0094092, Pat. Appl. Pub. No. US-2006-0115881-A1 and Pat. Appl. Pub. No. US-2006-0110806-A1. This list is not exhaustive and should not be construed as limiting.

[0126] Briefly, a variety of ω-3/ω-6 PUFA products can be produced prior to their transfer to TAGs, depending on the fatty acid substrate and the particular genes of the ω-3/ω-6 fatty acid biosynthetic pathway that are present in or transformed into the host cell. As such, production of the desired fatty acid product can occur directly or indirectly. Direct production occurs when the fatty acid substrate is converted directly into the desired fatty acid product without any intermediate steps or pathway intermediates. Indirect production occurs when multiple genes encoding the PUFA biosynthetic pathway may be used in combination such that a series of reactions occur to produce a desired PUFA. Specifically, it may be desirable to transform an oleaginous yeast with an expression cassette comprising a Δ12 desaturase, Δ6 desaturase, a $C_{18/20}$ elongase, a Δ5 desaturase and a Δ17 desaturase for the overproduction of EPA. See U.S. Pat. No. 7,238,482 and Int'l. App. Pub. No. WO 2006/052870. As is well known to one skilled in the art, various other combinations of genes encoding enzymes of the PUFA biosynthetic pathway may be useful to express in an oleaginous organism (see FIG. **1**). The particular genes included within a particular expression cassette depend on the host organism, its PUFA profile and/or desaturase/elongase profile, the availability of substrate and the desired end product(s).

[0127] A number of candidate genes having the desired desaturase and/or elongase activities can be identified according to publicly available literature, such as GenBank, the patent literature, and experimental analysis of organisms having the ability to produce PUFAs. Useful desaturase and elongase sequences may be derived from any source, e.g., isolated from a natural source such as from bacteria, algae, fungi, oomycete, yeast, plants, animals, etc., produced via a semi-synthetic route or synthesized de novo. Following the identification of these candidate genes, considerations for choosing a specific polypeptide having desaturase or elongase activity include: 1) the substrate specificity of the polypeptide; 2) whether the polypeptide or a component thereof is a rate-limiting enzyme; 3) whether the desaturase or elongase is essential for synthesis of a desired PUFA; 4) co-factors required by the polypeptide; and/or, 5) whether the polypeptide is modified after its production, such as by a kinase or a prenyltransferases.

[0128] The expressed polypeptide preferably has parameters compatible with the biochemical environment of its location in the host cell. See U.S. Pat. No. 7,238,482. It may also be useful to consider the conversion efficiency of each particular desaturase and/or elongase. More specifically, since each enzyme rarely functions with 100% efficiency to convert substrate to product, the final lipid profile of unpurified oils produced in a host cell is typically a mixture of various PUFAs consisting of the desired ω-3/ω-6 fatty acid, as well as various upstream intermediary PUFAs. Thus, the conversion efficiency of each enzyme is also a variable to consider when optimizing biosynthesis of a desired fatty acid.

Peroxisome Biogenesis and Pex Genes

[0129] As previously described, peroxisomes are ubiquitous organelles found in all eukaryotic cells. Their primary role is the degradation of various substances within a localized organelle of the cell, such as toxic compounds, fatty acids, etc. For example, the process of β-oxidation, wherein fatty acid molecules are broken down to ultimately produce free molecules of acetyl-CoA (which are exported back to the cytosol), can occur in peroxisomes. Although the process of β-oxidation in mitochondria results in ATP synthesis, β-oxidation in peroxisomes causes the transfer of high-potential electrons to O_2 and results in the formation of H_2O_2, which is subsequently converted to water and O_2 by peroxisome catalases. Very long chain, such as C_{18} to C_{22}, fatty acids undergo initial β-oxidation in peroxisomes, followed by mitochondrial β-oxidation.

[0130] The proteins responsible for importing proteins by means of ATP hydrolysis through the peroxisomal membrane are known as peroxisome biogenesis factor proteins, or "peroxins". These peroxisome biogenesis factor proteins also include those proteins involved in peroxisome biogenesis/assembly. The gene acronym for peroxisome biogenesis factor proteins is Pex; and, a system for nomenclature is described by Distel et al., *J. Cell Biol.*, 135:1-3 (1996). At least 32 different Pex genes have been identified so far in various eukaryotic organisms. In fungi, however, the recent review of Kiel et al. (*Traffic*, 7:1291-1303 (2006)) suggests that the minimal requirement for peroxisome biogenesis/matrix protein import is numbered as 17, thereby requiring only Pex1p, Pex2p, Pex3p, Pex4p, Pex5p, Pex6p, Pex7p, Pex8p, Pex10p, Pex12p, Pex13p, Pex14p, Pex17p, Pex19p, Pex20p, Pex22p and Pe26p. These proteins act in a coordinated fashion to proliferate (duplicate) peroxisomes and import proteins via translocation into peroxisomes (reviewed in Waterham, H. R. and J. M. Cregg. *BioEssays.* 19(1):57-66 (1996)).

[0131] Many Pex genes were initially isolated from the analysis of mutants that demonstrated abnormal peroxisomal functions or structures. With the availability of complete genome sequences, however, it is becoming increasingly easy to identify Pex genes via computer sequence searches based on homology. Kiel et al. (*Traffic*, 7:1291-1303 (2006)) cite strong conservation of the peroxisome biogenesis machinery, despite occasional low sequence similarity. More specifically, within the yeast and filamentous fungi, their data indicate that almost all Pex proteins identified thus far are conserved. Table 4, below, shows peroxisome biogenesis factor proteins identified by Kiel et al. (supra) in *Saccharomyces cerevisiae, Candida glabrata, Ashbya gossypii, Kluyveromyces lactis, Candida albicans, Debaryomyces hansenii, Pichia pastoris, Hansenula polymorpha, Yarrowia lipolytica, Aspergillus fumigatus, Aspergillus nidulans, Penicillium chrysogenum, Magnaporthe grisea, Neurospora crassa, Gibberella zeae, Ustilago maydis, Cryptococcus neoformans* var. *neoformans* and *Schizosaccharomyces pombe*.

TABLE 4

GenBank Accession Numbers Of Fungal Peroxisome Biogenesis Factor Proteins
[Recreated From Table 2 of Kiel et al., (Traffic, 7: 1291-1303 (2006))]

	Saccharo-myces cerevisiae	*Candida glabrata*	*Ashbya gossypii*	*Kluyveromyces* lactis	*Candida albicans*	*Debaryomyces hansenii*	*Pichia pastoris*	*Hansenula polymorpha*	*Yarrowia lipolytica*
Pex1p	CAA82041	CAG60131	AAS53742	CAH02218	EAL02496	CAG89689	CAA85450	AAD52811	CAG82178
Pex2p	CAA89508	CAG60461	AAS50677	CAH00186	EAK95929	CAG85956	CAA65646	AAT97412	CAG77647
Pex3p	AAB64764	CAG62379	AAS52217	CAG99801	EAK94771	CAG89890	CAA96530	AAC49471	CAG78565
Pex3Bp	—	—	—	—	—	—	na	—	CAG83356
Pex4p	CAA97146	CAG60639	AAS53685	CAG99212	EAL03336	CAG87262	AAA53634	AAC16238	CAG79130
Pex5p	CAA89730	CAG61665	AAS53824	CAH01742	EAK94251	CAG89098	AAB40613	AAC49040	CAG78803
Pex5Bp	—	CAG61076	—	—	—	—	na	—	—
Pex5Cp	CAA89120 (Ymr018wp)	—	—	—	—	—	na	—	—
Pex5/20p	—	—	—	—	—	—	na	—	—
Pex5Rp	—	—	—	—	—	—	na	—	—
Pex6p	AAA16574	CAG58438	AAS54884	CAG99125	EAK95956	CAG87108	CAA80278	AAD52812	CAG82306
Pex7p	CAA57183	CAG57936	AAS54301	CAG99215	EAK95226	CAG87150	AAC08303	ABA64462	CAG78389
Pex8p	CAA97079	CAG61238	AAS52889	CAH01253	EAK91777, EAK91778*	CAG89446	AAC41653	CAA82928	CAG80447
Pex9p	ORF wrongly identified	—	—	—	—	—	—	—	—
Pex10p	AAB64453	CAG62699	AAS53069	CAG99788	Translation of AACQ-01000128, nucleotides 37281-36306 (contains intron)	CAG89101	AAB09086	CAA86101	CAG81606
Pex12p	CAA89129	CAG62649	AAS50837	CAG99378	EAL00707	CAG84342	AAC49402	AAM66157	CAG81532
Pex13p	AAB46885	CAG57840	AAS51456	CAG99931	EAK97421	CAG86337	AAB09087	DQ345349	CAG81789
Pex14p	AAS56829	CAG58828	AAS54871	CAG99440	EAK90926	CAG91028	AAG28574	AAB40596	CAG79323
Pex15p	CAA99046	CAG58938	AAS51506	CAG98135	—	—	na	—	—
Pex16p	—	—	—	—	—	—	na	—	CAG79622
Pex17p	CAA96116	CAG61398	AAS50595	CAH01010	EAK95385	CAG86168	AAF19606	DQ345350	CAG84025
Pex14/17p	—	—	—	—	—	—	na	—	—
Pex18p	AAB68992	—	—	—	—	—	na	—	—
Pex19p	CAA98630	CAG58359	AAS52741	CAG99258	EAK97275	CAG84799	AAD43507	AAK84070	AAK84827
Pex20p	—	—	—	—	EAK91603, EAK94766*	CAG87898	AAX11696	AAX14715	CAG79226
Pex21p	CAA97267	CAG59241	AAS51769	CAG99735	—	—	na	—	—
Pex21Bp	—	CAG60281	—	—	—	—	na	—	—
Pex22p	AAC04978	CAG60970	AAS52329	CAG97800	EAK91040	CAG88727	AAD45664	DQ384616	CAG77876
Pex22p-like	—	—	—	—	—	na	—	—	EAL90994
Pex26p	—	—	—	—	EAK91093	CAG88929	na	DQ645588	Antisense translation of NC_006072, nucleotides 117230-118387

	Aspergillus fumigatus	*Aspergillus nidulans*	*Penicillium chrysogenum*	*Magnaporthe grisea*	*Neurospora crassa*	*Gibberella zeae*	*Ustilago maydis*	*Cryptococcus neoformans* var. *neoformans*	*Schizo-saccharomyces pombe*
Pex1p	EAL93310	EAA57740	AAG09748	XP_364454	EAA34641	EAA76787	EAK85195	AAW43248	CAA19256
Pex2p	EAL88068	EAA58944	DQ793192	XP_368589	EAA35361	EAA70670	EAK81310	AAW40683	CAA16981
Pex3p	EAL91965	EAA64392	DQ793193	XP_369909	EAA33751	EAA76989	EAK87104	AAW42444	CAB10141
Pex3Bp	—	—	—	—	—	—	—	—	—
Pex4p	EAL87211	Translation of AACD0-1000130, nucleotides 150195-150738 (contains intron)	DQ793194	XP_369064	EAA34737	EAA76379	Translation of AACP0-1000006, nucleotides 97041-96550 (contains intron)	—	CAB91184
Pex5p	EAL85289	EAA63772	AAR12222	XP_360528	EAA36111	EAA68640	EAK83659	AAW46349	CAA22179
Pex5Bp	—	—	—	—	—	—	—	—	—
Pex5Cp	—	—	—	—	—	—	—	—	—
Pex5/20p	—	—	—	—	—	—	EAK82973	AAW41849	—
Pex5Rp	—	—	—	—	—	—	—	—	—
Pex6p	EAL92776	EAA63496	AAG09749	XP_368715	EAA36040	EAA73732	EAK83459	AAW45333	CAB11501

TABLE 4-continued

GenBank Accession Numbers Of Fungal Peroxisome Biogenesis Factor Proteins
[Recreated From Table 2 of Kiel et al., (Traffic, 7: 1291-1303 (2006))]

Pex7p	EAL90870	EAA65909	DQ793195	XP_363555	AAN39560	EAA74171	EAK84499	AAW41119	P78798
Pex8p	EAL93137	EAA57947	DQ793196	XP_359449	EAA27783	EAA77627	EAK83936	AAW43468	CAB53406
Pex9p	—	—	—	—	—	—	—	—	—
Pex10p	EAL87045	EAA62774	DQ793197	XP_369099	EAA34967	EAA76761	EAK83811	AAW45079	CAB51769
Pex12p	EAL93972	EAA61357	DQ793198	XP_363845	EAA32773	EAA76413	EAK81282	AAW46724	CAD27496
Pex13p	EAL85282	EAA63824	DQ793199	XP_369087	EAA35785	EAA68396	EAK84395	AAW42381	CAB16740
Pex14p	EAL92562	EAA61046	DQ793200	XP_368216	EAA28304	EAA76904	EAK83123	AAW46857	CAA18656
Pex15p	—	—	—	—	—	—	—	—	—
Pex16p	EAL88469	EAA62294	DQ793201	XP_364166	EAA34648	EAA71849	EAK82801	AAW43797	CAA22819
Pex17p	See Pex14/17p	—	—	—	—	—	—	—	—
Pex14/17p	EAL93590	EAA58642	DQ793202	XP_368163	EAA27748	EAA73655	EAK81127	—	—
Pex18p	—	—	—	—	—	—	—	—	—
Pex19p	EAL92487	EAA60977	DQ793203	XP_368273	EAA31855	EAA70162	EAK86072	AAW42876	CAA97344
Pex20p	EAL90176	EAA60479	DQ793204	XP_368606	AAN39561	EAA76911	—	—	—
Pex21p	—	—	—	—	—	—	—	—	—
Pex21Bp	—	—	—	—	—	—	—	—	—
Pex22p	—	—	—	—	—	—	—	—	—
Pex22p-like	EAL90994	EAA66006	DQ793205	XP_365689	EAA26537	Translation of AACM0-1000080, nucleotides 4362-3039 (contains intron)	—	—	—
Pex26p	EAL93994	EAA61336	DQ793206	XP_359606	EAA28582	EAA76391	—	—	—

*Partial ORFs encoded on non-overlapping contigs.

[0132] Mutations of Pex genes leading to impaired peroxisome biogenesis result in severe metabolic and developmental disturbances in yeasts, humans and plants (Eckert, J. H. and R. Erdmann, *Rev. Physiol. Biochem Pharmacol.,* 147:75-121 (2003); Weller, S. et al., *Annual Review of Genomics and Human Genetics,* 4:165-211 (2003); Wanders, R. J., *Am. J. Med. Genet.,* 126A:355-375 (2004); Mano, S. and M. Nishimura, *Vitam Horm.,* 72:111-154 (2005); Wanders, J. A., and H. R. Waterham, *Annu. Rev. Biochem.,* 75:295-332 (2006); Fujiki, Yukio. Peroxisome Biogenesis Disorders. In, *Encyclopedia of Life Sciences*. John Wiley & Sons, 2006). For example, X-linked adrenoleukodystrophy ["X-ALD"] and Zellweger syndrome, as well as several less severe forms of the disease, can result from single enzyme deficiencies and/or peroxisomal biogenesis disorders.

[0133] Within the yeast, *Yarrowia lipolytica*, a variety of different Pex genes have been isolated and characterized, as identified in Table 4 above. More specifically, Bascom, R. A. et al. (*Mol. Biol. Cell,* 14:939-957 (2003)) describe YIPex3p; Szilard, R. K. et al. (*J. Cell Biol.,* 131:1453-1469 (1995)) describe YIPex5p; Nuttley, W. M. et al. (*J. Biol. Chem.,* 269: 556-566 (1994)) describe YIPex6p; Elizen G. A., et al. (*J. Biol. Chem.,* 270:1429-1436 (1995)) describe YIPex9p; Elizen G. A., et al. (*J. Cell Biol.,* 137:1265-1278 (1997)) and Titorenko, V. I. et al. (*Mol. Cell. Biol.,* 17:5210-5226 (1997)) describe YIPex16p; Lambkin, G. R. and R. A. Rachubinski (*Mol. Biol. Cell.,* 12(11):3353-3364 (2001)) describe YIPex19; and Titorenko V. I., et al. (*J. Cell Biol.,* 142:403-420 (1998)) and Smith J. J. and R. A. Rachubinski (*J. Cell Biol.,* 276:1618-1625 (2001)) describe YIPex20p.

[0134] Of initial interest herein was YIPex10p (GenBank Accession No. CAG81606, No. AB036770 and No. AJ012084). It was demonstrated in Sumita et al. (*FEMS Microbiol. Lett.,* 214:31-38 (2002) that: 1) YIPex10p functions as a component of the peroxisome; and, 2) the C_3HC_4 zinc ring finger motif of YIPex10p was essential for the protein's function as determined via creation of C341S, C346S and H343W point mutations, followed by analysis of growth.

[0135] Studies of the C_3HC_4 zinc ring finger motif of Pex10 have been done in other organisms with similar results. For example, point mutations that alter conserved residues in the Pex10p C_3HC_4 motif of *Pichia pastoris* were found to abolish function of the protein (Kalish, J. E. et al., *Mol. Cell. Biol.,* 15:6406-6419 (1995)). Similarly, after functional complementation assays in fibroblast cell lines, Warren D. S., et al. (*Hum. Mutat.,* 15(6):509-521 (2000)) concluded that the C_3HC_4 motif was critical for Pex10p function. Several studies show that loss of function of Pex10p in *Arabidopsis* causes embryo lethality at the heart stage (Hu, J., et al., *Science,* 297:405-409 (2002); Schmumann, U. et al., *Proc. Natl. Acad. Sci. U.S.A.,* 100:9626-9631 (2003); Sparkes, I. A., et al., *Plant Physiol.,* 133:1809-1819 (2003); Fan, J. et al., *Plant Physiol.,* 139:231-239 (2005)). In follow-up research, Schemann, U. et al. (*Proc. Natl. Acad. Sci. U.S.A.,* 104:1069-1074 (2007)) investigated the function of Pex10p in nonlethal partial loss-of-function *Arabidopsis* mutants. Specifically, four T-DNA insertion lines expressing Pex10p with a dysfunctional C_3HC_4 motif were created in an *Arabidopsis* wildtype background. Mutant plants demonstrated impaired leaf peroxisomes and the authors suggest that inactivation of the ring finger motif in Pex10p eliminated protein interaction required for attachment of peroxisomes to chloroplasts and movement of metabolites between peroxisomes and chloroplasts.

[0136] Although studies have not identified essential domains in other Pex proteins, research has looked at the effect of various Pex mutants to learn the strategies and the molecular mechanisms evolutionarily diverse organisms use for assembling, maintaining, propagating and inheriting the peroxisome, an organelle known for its role in lipid metabolism. For example, Bascom, R. A. et al. has performed knock-

out and overexpression of the *Yarrowia lipolytica* Pex3p (*Mol. Biol. Cell,* 14:939-957 (2003)). The knockout cells did not contain wildtype perixosomes but instead had numerous small vesicles; overexpression resulted in cells with fewer, larger and clustered peroxisomes. They hypothesized that Pex3p is involved in the initiation of peroxisome assembly by sequestering components of peroxisome biogenesis, i.e., peroxisome targeting signal (PTS) 1 and 2 import machineries. Similarly, for Guo, T. et al., knockout of the *Y lipolytica* Pex16p resulted in excessive proliferation of immature peroxisomal vesicles and significantly decreased the rate and efficiency of their conversion to mature peroxisomes (*J. Cell Biol.,* 162:1255-1266 (2003)), while overexpression resulted in few but enlarged peroxisomes (Eitzen et al., *J. Cell Biol.,* 137:1265-1278 (1997)). Guo et al. concluded Pex16p negatively regulated the membrane scission event required for division of early peroxisomal precursors.

[0137] Despite the advances summarized above, many details concerning the roles of various Pex proteins, their interaction with one another and the biogenesis/assembly mechanism in peroxisomes remains to be elucidated. As such, the data described in the Application, wherein mutation within the C_3HC_4 motif of YlPex10p or knockout of YlPex3p, YlPex10p or YlPex16p results in creation of a *Yarrowia lipolytica* mutant that has an increased capacity to incorporate PUFAs, especially long-chain PUFAs such as C_{20} to C_{22} molecules, into the total lipid fraction and in the oil fraction in the cell, is a novel observation that does not yet find validation in studies with other plants or animals.

[0138] It has been suggested that peroxisomes are required for both catabolic and anabolic lipid metabolism (Lin, Y. et al., *Plant Physiology,* 135:814-827 (2004)); however, this hypothesis was based on studies with a homolog of Pex16p. More specifically, Lin, Y. et al. (supra) reported that *Arabidopsis Shrunken Seed* 1 (sse1) mutants had both abnormal peroxisome biogenesis and fatty acid synthesis, based on a reduction of oil to approximately 10-16% of wild type in sse1 seeds. Binns, D. et al. (*J. Cell Biol.,* 173(5):719-731 (2006)) examined the peroxisome-lipid body interactions in *Saccharomyces cerevisiae* and determined that extensive physical contact between the two organelles promotes coupling of lipolysis within lipid bodies with peroxisomal fatty acid oxidation. More specifically, ratios of free fatty acids to TAGs were examined in various Pex knockouts and found to be increased relative to the wildtype. Clearly, further investigation will be necessary to understand the metabolic roles of peroxisomes and in particular of Pex3p, Pex10p and Pex16p proteins.

[0139] Without wishing to be held to any particular explanation or theory, it is hypothesized that disruption or knockout of a Pex gene within an oleaginous yeast cell affects both the catabolic and anabolic lipid metabolism that naturally occurs in peroxisomes or is affected by peroxisomes. Disruption or knockout results in an increase in the amount of PUFAs in the total lipid fraction and in the oil fraction, as a percent of total fatty acids, as compared with an oleaginous yeast whose native peroxisome biogenesis factor protein has not been disrupted. In some cases, an increase in the amount of PUFAs in the total lipid fraction and in the oil fraction as a percent of dry cell weight, and/or an increase in the total lipid content as a percent of dry cell weight, is also observed. It is hypothesized that this generalized mechanism is applicable within all eukaryotic organisms, such as algae, fungi, oomycetes, yeast, euglenoids, stramenopiles, plants and some mammalian systems, since all comprise peroxisomes.

[0140] Identification and Isolation of Pex Homologs

[0141] When the sequence of a particular Pex gene or protein within a preferred host organism is not known, one skilled in the art recognizes that it will be most desirable to identify and isolate these genes, or portions of them, prior to regulating the activity of the encoded proteins, which regulation in turn facilitates altering the amount, as a percent of total fatty acids, of PUFAs incorporated into the total lipid fraction and in the oil fraction of the eukaryote. Sequence knowledge of the preferred host's Pex genes also facilitates disruption of the homologous chromosomal genes by targeted disruption.

[0142] The Pex sequences in Table 4, or portions of them, may be used to search for Pex 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 Pex protein in Table 4 against a database of nucleic or protein sequences and thereby identifying similar known sequences within a preferred host organism.

[0143] 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 Pex sequences, such as those described in Table 4. It is predictable that isolation would be relatively easier for Pex homologs of at least about 70%-85% identity to publicly available Pex 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.

[0144] Some Pex homologs have also been isolated by the use of motifs unique to the Pex enzymes. For example, it is well known that Pex2p, Pex10p and Pex12p all share a cysteine-rich motif near their carboxyl termini, known as a C_3HC_4 zinc ring finger motif (FIG. **2**A). This region of "conserved domain" corresponds to a set of amino acids that are highly conserved at specific positions and likely represents a region of the Pex protein that is essential to the structure, stability or activity of the protein. Motifs are identified by their high degree of conservation in aligned sequences of a family of protein homologues. As unique "signatures", they can determine if a protein with a newly determined sequence belongs to a previously identified protein family. These motifs are useful as diagnostic tools for the rapid identification of novel Pex2, Pex10 and/or Pex12 genes, respectively.

[0145] Alternatively, the publicly available Pex sequences or their motifs may be hybridization reagents for the identification of homologs. The basic components of a nucleic acid hybridization test include a probe, a sample suspected of containing the gene or gene fragment of interest, and a specific hybridization method. Probes are typically single-stranded nucleic acid sequences that are complementary to the nucleic acid sequences to be detected. Probes are hybridizable to the nucleic acid sequence to be detected. Although probe length can vary from 5 bases to tens of thousands of bases, typically a probe length of about 15 bases to about 30 bases is suitable. Only part of the probe molecule need be

complementary to the nucleic acid sequence to be detected. In addition, the complementarity between the probe and the target sequence need not be perfect. Hybridization does occur between imperfectly complementary molecules with the result that a certain fraction of the bases in the hybridized region are not paired with the proper complementary base.

[0146] Hybridization methods are well known. Typically the probe and the sample must be mixed under conditions that permit nucleic acid hybridization. This involves contacting the probe and sample in the presence of an inorganic or organic salt under the proper concentration and temperature conditions. The probe and sample nucleic acids must be in contact for a long enough time that any possible hybridization between the probe and the sample nucleic acid occurs. The concentration of probe or target in the mixture determine the time necessary for hybridization to occur. The higher the concentration of the probe or target, the shorter the hybridization incubation time needed. Optionally, a chaotropic agent may be added, such as guanidinium chloride, guanidinium thiocyanate, sodium thiocyanate, lithium tetrachloroacetate, sodium perchlorate, rubidium tetrachloroacetate, potassium iodide or cesium trifluoroacetate. If desired, one can add formamide to the hybridization mixture, typically 30-50% (v/v) ["by volume"].

[0147] Various hybridization solutions can be employed. Typically, these, comprise from about 20 to 60% volume, preferably 30%, of a polar organic solvent. A common hybridization solution employs about 30-50% v/v formamide, about 0.15 to 1 M sodium chloride, about 0.05 to 0.1 M buffers (e.g., sodium citrate, Tris-HCl, PIPES or HEPES (pH range about 6-9)), about 0.05 to 0.2% detergent (e.g., sodium dodecylsulfate), or between 0.5-20 mM EDTA, FICOLL (Pharmacia Inc.) (about 300-500 kdal), polyvinylpyrrolidone (about 250-500 kdal), and serum albumin. Also included in the typical hybridization solution are unlabeled carrier nucleic acids from about 0.1 to 5 mg/mL, fragmented nucleic DNA such as calf thymus or salmon sperm DNA or yeast RNA, and optionally from about 0.5 to 2% wt/vol ["weight by volume"] glycine. Other additives may be included, such as volume exclusion agents that include polar water-soluble or swellable agents (e.g., polyethylene glycol), anionic polymers (e.g., polyacrylate or polymethylacrylate) and anionic saccharinic polymers, such as dextran sulfate.

[0148] Nucleic acid hybridization is adaptable to a variety of assay formats. One of the most suitable is the sandwich assay format. The sandwich assay is particularly adaptable to hybridization under non-denaturing conditions. A primary component of a sandwich-type assay is a solid support. The solid support has adsorbed or covalently coupled to it immobilized nucleic acid probe that is unlabeled and complementary to one portion of the sequence.

[0149] Any of the Pex nucleic acid fragments or any identified homologs may be used to isolate genes encoding homologous proteins from the same or other algal, fungal, oomycete, euglenoid, stramenopiles, yeast or plant species. 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, S. 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.

[0150] For example, genes encoding proteins or polypeptides similar to publicly available Pex genes or their motifs could be isolated directly by using all or a portion of those publicly available nucleic acid fragments as DNA hybridization probes to screen libraries from any desired organism using well known methods. Specific oligonucleotide probes based upon the publicly available nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis, supra). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan, such as random primers DNA labeling, nick translation or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part or the full length of the publicly available sequences or their motifs. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full-length DNA fragments under conditions of appropriate stringency.

[0151] Typically, in PCR-type amplification techniques, the primers have different sequences and are not complementary to each other. Depending on the desired test conditions, the sequences of the primers should be designed to provide for both efficient and faithful replication of the target nucleic acid. Methods of PCR primer design are common and well known (Thein and Wallace, "The use of oligonucleotides as specific hybridization probes in the Diagnosis of Genetic Disorders", in *Human Genetic Diseases: A Practical Approach*, K. E. Davis Ed., (1986) pp 33-50, IRL: Herndon, Va.; Rychlik, W., In *Methods in Molecular Biology*, White, B. A. Ed., (1993) Vol. 15, pp 31-39, PCR Protocols: Current Methods and Applications. Humania: Totowa, N.J.).

[0152] Generally two short segments of available Pex sequences may be used in PCR protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. PCR may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the available nucleic acid fragments or their motifs. The sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding genes.

[0153] Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al., *Proc. Natl. Acad. Sci. U.S.A.,* 85:8998 (1988)) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the available sequences. Using commercially available 3' RACE or 5' RACE systems (e.g., BRL, Gaithersburg, Md.), specific 3' or 5' cDNA fragments can be isolated (Ohara et al., *Proc. Natl. Acad. Sci. U.S.A.,* 86:5673 (1989); Loh et al., *Science,* 243:217 (1989)).

[0154] Based on any of these well-known methods just discussed, it would be possible to identify and/or isolate Pex gene homologs in any preferred eukaryotic organism of choice. The activity of any putative Pex gene can readily be confirmed by targeted disruption of the endogenous gene within the PUFA-producing host organism, since the lipid

profiles of the total lipid fraction and of the oil fraction are modified relative to those within an organism lacking the targeted Pex gene disruption.

Increasing the Amount of PUFAs in the Total Lipid Fraction and in the Oil Fraction Via Disruption of a Native Peroxisome Biogenesis Factor Protein

[0155] As noted above, the present disclosure relates to the following described methods for increasing the weight percent of one PUFA or a combination of PUFAs in an oleaginous eukaryotic organism, comprising:

[0156] a) providing an oleaginous eukaryotic organism comprising a disruption in a native gene encoding a peroxisome biogenesis factor protein, which creates a PEX-disruption organism; and genes encoding a functional PUFA biosynthetic pathway; and,

[0157] b) growing the eukaryotic organism of (a) under conditions wherein the weight percent of one PUFA or a combination of PUFAs is increased in the total lipid fraction and in the oil fraction relative to the weight percent of the total fatty acids, when compared with those weight percents in an oleaginous eukaryotic organism whose native peroxisome biogenesis factor protein has not been disrupted.

The amount of PUFAs that increases as a percent of total fatty acids can be: 1) the PUFA that is the desired end product of a functional PUFA biosynthetic pathway, as opposed to PUFA intermediates or by-products; 2) C_{20} to C_{22} PUFAs; and/or, 3) total PUFAs.

[0158] In addition to the increase in the weight percent of one or a combination of PUFAs relative to the weight percent of the total fatty acids, in some cases, the total lipid content (TFA % DCW) of the cell may be increased or decreased. What this means is that regardless of whether the disruption in the PEX gene causes the amount of total lipids in the PEX-disrupted cell to increase or decrease, the disruption always causes the weight percent of a PUFA or of a combination of PUFAs to increase.

[0159] Another method provided herein relates to a disruption in a native gene encoding a peroxisome biogenesis factor protein, wherein said disruption can result in an increase in the percent of one PUFA or a combination of PUFAs relative to the dry cell weight when compared to that percent in a parental strain whose native Pex protein had not been disrupted or that was expressing a "replacement" copy of the disrupted native Pex protein.

[0160] In preferred aspects of the method above, the disruption in a native gene encoding a peroxisome biogenesis factor protein results in an increase in the amount of the PUFA that is the desired end product of a functional PUFA biosynthetic pathway, as opposed to PUFA intermediates or by-products, as a percent of dry cell weight relative to the parental strain whose native Pex protein had not been disrupted or the parental strain that was expressing a "replacement" copy of the disrupted native Pex protein. In some cases, the increase in the percent of a combination of PUFAs relative to the dry cell weight is a combination of C_{20} to C_{22} PUFAs or the total PUFAs.

[0161] Also described herein are organisms produced by these methods, comprising a disruption of at least one peroxisome biogenesis factor protein. Lipids and oils obtained from these organisms, products obtained from the processing of the lipids and oil, use of these lipids and oil in foods, animal feeds or industrial applications and/or use of the by-products in foods or animal feeds are also described.

[0162] Preferred eukaryotic organisms in the methods described above include algae, fungi, oomycetes, yeast, euglenoids, stramenopiles, plants and some mammalian systems.

[0163] The peroxisome biogenesis factor protein for any of these methods may be selected from the group consisting of: Pex1p, Pex2p, Pex3p, Pex3Bp Pex4p, Pex5p, Pex5Bp, Pex5Cp, Pex5/20p, Pex6p, Pex7p, Pex8p, Pex10p, Pex12p, Pex13p, Pex14p, Pex15p, Pex16p, Pex17p, Pex14/17p, Pex18p, Pex19p, Pex20p, Pex21p, Pex21B, Pex22p, Pex22p-like and Pex26p (and protein homologs thereof). In some preferred methods described herein, the disrupted peroxisome biogenesis factor protein is selected from the group consisting of: Pex2p, Pex3p, Pex10p, Pex12p and/or Pex16p. In some more preferred methods, however, the disrupted peroxisome biogenesis factor protein is selected from the group consisting of: Pex3p, Pex10p and/or Pex16p.

[0164] The disruption in the native gene encoding a peroxisome biogenesis factor protein can be an insertion, deletion, or targeted mutation within a portion of the gene, such as within the N-terminal portion of the protein or within the C-terminal portion of the protein. Alternatively, the disruption can result in a complete gene knockout such that the gene is eliminated from the host cell genome. Or, the disruption could be a targeted mutation that results in a non-functional protein.

Disruption Methodologies

[0165] The invention includes disruption in a native gene encoding a peroxisome biogenesis factor protein within a preferred host cell. Although numerous techniques are available to one of skill in the art to achieve disruption, generally the endogenous activity of a particular gene can be reduced or eliminated by the following techniques, for example: 1) disrupting the gene through insertion, substitution and/or deletion of all or part of the target gene; or 2) manipulating the regulatory sequences controlling the expression of the protein. Both of these techniques are discussed below. However, one skilled in the art appreciates that these are well described in the existing literature and are not limiting to the methods, host cells, and products described herein. One skilled in the art also appreciates the most appropriate technique for use with any particular oleaginous yeast.

[0166] Disruption Via Insertion, Substitution And/Or Deletion: For gene disruption, a foreign DNA fragment, typically a selectable marker gene, is inserted into the structural gene. This interrupts the coding sequence of the structural gene and causes inactivation of that gene. Transformation of the disruption cassette into the host cell results in replacement of the functional native gene by homologous recombination with the non-functional disrupted gene. See, for example: Hamilton et al., *J. Bacteriol.,* 171:4617-4622 (1989); Balbas et al., *Gene,* 136:211-213 (1993); Gueldener et al., *Nucleic Acids Res.,* 24:2519-2524 (1996); and Smith et al., *Methods Mol. Cell. Biol.,* 5:270-277 (1996). One skilled in the art appreciates the many variations of the general method of gene targeting, which admits of positive or negative selection, creation of gene knockouts, and insertion of exogenous DNA sequences into specific genome sites in mammalian systems, plant cells, filamentous fungi, algae, oomycetes, euglenoids, stramenopiles, yeast and/or microbial systems.

[0167] In contrast, a non-specific method of gene disruption is the use of transposable elements or transposons. Transposons are genetic elements that insert randomly into DNA but can be later retrieved on the basis of sequence to determine the locus of insertion. Both in vivo and in vitro transposition techniques are known and involve the use of a transposable element in combination with a transposase enzyme. When the transposable element or transposon is contacted with a nucleic acid fragment in the presence of the transposase, the transposable element randomly inserts into the nucleic acid fragment. The technique is useful for random mutagenesis and for gene isolation, since the disrupted gene may be identified on the basis of the sequence of the transposable element. Kits for in vitro transposition are commercially available and include: the Primer Island Transposition Kit, available from Perkin Elmer Applied Biosystems, Branchburg, N.J., based upon the yeast Ty1 element; the Genome Priming System, available from New England Biolabs, Beverly, Mass., based upon the bacterial transposon Tn7; and EZ::TN Transposon Insertion Systems, available from Epicentre Technologies, Madison, Wis., based upon the Tn5 bacterial transposable element.

[0168] Manipulation Of Pex Regulatory Sequences: As is well known in the art, the regulatory sequences associated with a coding sequence include transcriptional and translational "control" nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of the coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Thus, manipulation of a Pex gene's regulatory sequences may refer to manipulation of the promoters, silencers, 5' untranslated leader sequences (between the transcription start site and the translation initiation codon), introns, enhancers, initiation control regions, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures of the particular Pex gene. In all cases, however, the result of the manipulation is down-regulation of the Pex gene's expression, which promotes increased amount of PUFAs in the total lipid fraction and in the oil fraction, as a percent of total fatty acids, as compared with an oleaginous yeast whose native peroxisome biogenesis factor protein has not been disrupted.

[0169] For example, the promoter of a Pex10 gene could be deleted or disrupted. Alternatively, the native promoter driving expression of a Pex10 gene may be substituted with a heterologous promoter having diminished promoter activity with respect to that of the native promoter. Methods useful for manipulating regulatory sequences are well known.

[0170] The skilled person is able to use these and other well known techniques to disrupt a native peroxisome biogenesis factor protein within the preferred host cells described herein, such as mammalian systems, plant cells, filamentous fungi, algae, oomycetes, euglenoids, stramenopiles and yeast.

[0171] One skilled in the art is able to discern the optimum means to disrupt the native Pex gene to achieve an increased amount of PUFAs that accumulate in the total lipid fraction and in the oil fraction, as a percent of total fatty acids, as compared with a eukaryotic organisms whose native peroxisome biogenesis factor protein has not been disrupted.

Metabolic Engineering of ω-3 and/or ω-6 Fatty Acid Biosynthesis

[0172] In addition to the methods described herein for disruption of a native peroxisome biogenesis factor protein, it may also be useful to manipulate ω-3 and/or ω-6 fatty acid biosynthesis. This may require metabolic engineering directly within the PUFA biosynthetic pathway or additional manipulation of pathways that contribute carbon to the PUFA biosynthetic pathway.

[0173] Techniques useful for up-regulating desirable biochemical pathways and down-regulating undesirable biochemical pathways are well known in the art. For example, biochemical pathways competing with the ω/-3 and/or ω-6 fatty acid biosynthetic pathways for energy or carbon, or native PUFA biosynthetic pathway enzymes that interfere with production of a particular PUFA end-product, may be eliminated by gene disruption or down-regulated by other means, such as antisense mRNA and zinc-finger targeting technologies.

[0174] The following discuss altering the PUFA biosynthetic pathway as a means to increase GLA, ARA, EPA or DHA, respectively, and desirable manipulations in the TAG biosynthetic pathway and in the TAG degradation pathway: Int'l. App. Pub. No. WO 2006/033723, Int'l. App. Pub. No. WO 2006/055322 [U.S. Pat. Appl. Pub. No. 2006-0094092-A1], Int'l. App. Pub. No. WO 2006/052870 [U.S. Pat. Appl. Pub. No. 2006-0115881-A1] and Int'l. App. Pub. No. WO 2006/052871 [U.S. Pat. Appl. Pub. No. 2006-0110806-A1], respectively.

Expression Systems, Cassettes, Vectors and Transformation of Host Cells

[0175] It may be necessary to create and introduce a recombinant construct into the preferred eukaryotic host, such as e.g., mammalian systems, plant cells, filamentous fungi, algae, oomycetes, euglenoids, stramenopiles and yeast, to result in disruption of a native peroxisome biogenesis factor protein and/or introduction of genes encoding a PUFA biosynthetic pathway. One of skill in the art appreciates 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 Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2^{nd} ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y. (1989); Maliga et al., Methods in Plant Molecular Biology, Cold Spring Harbor, N.Y. (1995); Birren et al., Genome Analysis: Detecting Genes, v. 1, Cold Spring Harbor, N.Y. (1998); Birren et al., Genome Analysis Analyzing DNA, v. 2, Cold Spring Harbor: NY (1998); *Plant Molecular Biology: A Laboratory Manual*, Clark, ed. Springer: NY (1997).

[0176] In general, the choice of sequences included in the 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. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector to successfully transform, select and propagate host cells containing the chimeric gene. Typically, however, the vector or cassette contains sequences directing transcription and translation of the relevant gene(s), a selectable marker and sequences allowing autonomous replication or chromosomal integration. Suitable vectors comprise a region 5' of the gene that controls transcriptional initiation, i.e., a promoter, and a region 3' of the DNA fragment that controls transcriptional termination, i.e., a terminator. It is most preferred when both control regions are derived from genes from the transformed host cell.

[0177] Initiation control regions or promoters useful for driving expression of heterologous genes or portions of them in the desired host cell are numerous and well known. These control regions may comprise a promoter, enhancer, silencer, intron sequences, 3' UTR and/or 5' UTR regions, and protein and/or RNA stabilizing elements. Such elements may vary in their strength and specificity. Virtually any promoter (i.e., native, synthetic, or chimeric) capable of directing expression of these genes in the selected host cell is suitable. 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 Pex gene of interest. Constitutive expression occurs by the use of a constitutive promoter operably linked to the gene of interest.

[0178] When the host cell is, for example, yeast, transcriptional and translational regions functional in yeast cells are provided, particularly from the host species. See Int'l. App. Pub. No. WO 2006/052870 for preferred transcriptional initiation regulatory regions for use in *Yarrowia lipolytica*. Any of a number of regulatory sequences may be used, depending on whether constitutive or induced transcription is desired, the efficiency of the promoter in expressing the ORF of interest, the ease of construction, etc.

[0179] 3' non-coding sequences encoding transcription termination signals, i.e., a "termination region", must be provided in a recombinant construct and may be from the 3' region of the gene from which the initiation region 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 termination region is selected more for convenience rather than for any particular property. Termination regions may also be derived from various genes native to the preferred hosts.

[0180] Particularly useful termination regions for use in yeast are those derived from a yeast gene, particularly *Saccharomyces, Schizosaccharomyces, Candida, Yarrowia* or *Kluyveromyces*. The 3'-regions of mammalian genes encoding γ-interferon and α-2 interferon are also known to function in yeast. The 3'-region can also be synthetic, as one of skill in the art can utilize available information to design and synthesize a 3'-region sequence that functions as a transcription terminator. A termination region may be unnecessary, but is highly preferred.

[0181] The vector may comprise a selectable and/or scorable marker, in addition to the regulatory elements described above. Preferably, the marker gene is an antibiotic resistance gene such that treating cells with the antibiotic causes inhibition of growth, or death, of untransformed cells and uninhibited growth of transformed cells. For selection of yeast transformants, any marker that functions in yeast is useful with resistance to kanamycin, hygromycin and the amino glycoside G418 and the ability to grow on media lacking uracil, lysine, histine or leucine being particularly useful.

[0182] Merely inserting a gene into a cloning vector does not ensure its expression at the desired rate, concentration, amount, etc. In response to the need for a high expression rate, many specialized expression vectors have been created by manipulating a number of different genetic elements that control transcription, RNA stability, translation, protein stability and location, oxygen limitation, and secretion from the host cell. Some of the manipulated features include: the nature of the relevant transcriptional promoter and terminator sequences, the number of copies of the cloned gene and whether the gene is plasmid-borne or integrated into the genome of the host cell, the final cellular location of the synthesized foreign 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. Each of these may be used in the methods and host cells described herein to further optimize expression of PUFA biosynthetic pathway genes and to diminish expression of a native Pex gene.

[0183] After a recombinant construct is created, e.g., comprising a chimeric gene comprising a promoter, ORF and terminator, suitable for disruption or knock out of a native peroxisome biogenesis factor protein and/or expression of genes encoding a PUFA biosynthetic pathway activity, it is placed in a plasmid vector capable of autonomous replication in the host cell or 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.

[0184] When two or more genes are expressed from separate replicating vectors, each vector may have a different means of selection and should lack homology to the other construct(s) to maintain stable expression and prevent reassortment of elements among constructs. Judicious choice of regulatory regions, selection means and method of propagation of the introduced construct(s) can be experimentally determined so that all introduced genes are expressed at the necessary levels to provide for synthesis of the desired products.

[0185] Constructs comprising the gene of interest may be introduced into a host cell by any standard technique. These techniques include transformation, e.g., lithium acetate transformation (*Methods in Enzymology,* 194:186-187 (1991)), protoplast fusion, biolistic impact, electroporation, microinjection, vacuum filtration or any other method that introduces the gene of interest into the host cell.

[0186] For convenience, a host cell that has been manipulated by any method to take up a DNA sequence, for example, in an expression cassette, is referred to herein as "transformed" or "recombinant". The transformed host will have at least one copy of the expression construct and may have two or more, depending upon whether the gene is integrated into the genome, amplified, or is present on an extrachromosomal element having multiple copy numbers.

[0187] 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. Typically, transformed hosts are selected for their ability to grow on selective media. Selective media may incorporate an antibiotic or lack a factor necessary for growth of the untransformed host, such as a nutrient or growth factor. An introduced marker gene may confer antibiotic resistance, or encode an essential growth factor or enzyme, thereby permitting growth on selective media when expressed in the transformed host. Selection of a transformed host can also occur when the expressed marker protein can be detected, either

directly or indirectly. The marker protein may be expressed alone or as a fusion to another protein. The marker protein can be detected by its enzymatic activity (e.g., β-galactosidase can convert the substrate X-gal ["5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside"] to a colored product; luciferase can convert luciferin to a light-emitting product) or its light-producing or modifying characteristics (e.g., the green fluorescent protein of *Aequorea Victoria* fluoresces when illuminated with blue light). Alternatively, antibodies can be used to detect the marker protein or a molecular tag on, for example, a protein of interest. Cells expressing the marker protein or tag can be selected, for example, visually, or by techniques such as fluorescence-activated cell sorting or panning using antibodies.

[0188] Regardless of the selected host or expression construct, multiple transformants must be screened to obtain a strain or plant line displaying the desired expression level, regulation and pattern, as different independent transformation events 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)). Such screening may be accomplished by 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 and/or Elisa analyses of protein expression, phenotypic analysis or GC analysis of the PUFA products.

Preferred Eukaryotic Host Organisms

[0189] A variety of eukaryotic organisms are suitable as host herein, to thereby yield a transformant host organism comprising a disruption in a native peroxisome biogenesis factor protein and genes encoding a PUFA biosynthetic pathway, wherein the transformed eukaryotic host organism has an increased amount of PUFAs incorporated into the total lipid fraction and in the oil fraction, as a percent of total fatty acids, as compared to a eukaryotic organism whose native peroxisome biogenesis factor protein has not been disrupted. Various mammalian systems, plant cells, fungi, algae, oomycetes, yeasts, stramenopiles and/or euglenoids may be useful hosts. Although oleaginous organisms are preferred, non-oleaginous organisms also have utility herein such that, when one of their native PEX genes is disrupted, an increase in the weight percent of at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids in the total lipid fraction or in the oil fraction will be experienced and may lead to a 1.3 fold increase in the PUFA. Additionally, the percent of the PUFA may be increased relative to the dry cell weight in the non-oleaginous organism. In alternate embodiments, a non-oleaginous organism can be genetically modified to become oleaginous, e.g., yeast such as *Saccharomyces cerevisiae.*

[0190] Oleaginous organisms are naturally capable of oil synthesis and accumulation, wherein the total oil content typically comprises greater than about 25% of the cellular dry weight. Various algae, moss, fungi, yeast, stramenopiles and plants are naturally classified as oleaginous.

[0191] Preferred oleaginous microbes include those algal, stramenopile and fungal organisms that naturally produce ω-3/ω-6 PUFAs. For example, ARA, EPA and/or DHA is produced via *Cyclotella* sp., *Nitzschia* sp., *Pythium, Thraustochytrium* sp., *Schizochytrium* sp. and *Mortierella.* 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.

[0192] More preferred are oleaginous yeast, including those that naturally produce and those genetically engineered to produce ω-3/ω-6 PUFAs. 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 yeasts 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*).

[0193] 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)).

[0194] Specific teachings relating to transformation of *Yarrowia lipolytica* include U.S. Pat. No. 4,880,741 and U.S. Pat. No. 5,071,764 and Chen, D. C. et al. (*Appl. Microbiol. Biotechnol.,* 48(2):232-235 (1997)), while suitable selection techniques are described in U.S. Pat. No. 7,238,482 and Int'l. App. Pub. Nos. WO 2005/003310 and WO 2006/052870.

[0195] The preferred method of expressing genes in *Yarrowia lipolytica* is by integration of linear DNA into the genome of the host. Integration into multiple locations within the genome can be particularly useful when high level expression of genes are desired, such as in the Ura3 locus (GenBank Accession No. AJ306421), the Leu2 gene locus (GenBank Accession No. AF260230), the Lys5 gene locus (GenBank Accession No. M34929), the Aco2 gene locus (GenBank Accession No. AJ001300), the Pox3 gene locus (Pox3: GenBank Accession No. XP_503244 or Aco3: GenBank Accession No. AJ001301), the Δ12 desaturase gene locus (U.S. Pat. No. 7,214,491), the Lip1 gene locus (GenBank Accession No. Z50020), the Lip2 gene locus (GenBank Accession No. AJ012632), the SCP2 gene locus (GenBank Accession No. AJ431362), the Pex3 gene locus (GenBank Accession No. CAG78565), the Pex16 gene locus (GenBank Accession No. CAG79622) and/or the Pex10 gene locus (GenBank Accession No. CAG81606).

[0196] Preferred selection methods for use in *Yarrowia lipolytica* are resistance to kanamycin, hygromycin and the amino glycoside G418, as well as ability to grow on media lacking uracil, leucine, lysine, tryptophan or histidine. 5-fluoroorotic acid [5-fluorouracil-6-carboxylic acid monohydrate or "5-FOA"] may also be used for selection of yeast Ura^- mutants. This compound is toxic to yeast cells that possess a functioning URA3 gene encoding orotidine 5'-monophosphate decarboxylase [OMP decarboxylase]; thus, based on this toxicity, 5-FOA is especially useful for the selection and identification of Ura^- mutant yeast strains (Bartel, P. L. and Fields, S., Yeast 2-Hybrid System, Oxford University: New York, v. 7, pp 109-147, 1997; see also Int'l. App. Pub. No. WO 2006/052870 for 5-FOA use in *Yarrowia*).

[0197] An alternate preferred selection method for use in *Yarrowia* relies on a dominant, non-antibiotic marker for *Yarrowia lipolytica* based on sulfonylurea (chlorimuron ethyl; E. I. duPont de Nemours & Co., Inc., Wilmington, Del.) resistance. More specifically, the marker gene is a native acetohydroxyacid synthase ("AHAS" or acetolactate syn-

thase; E.C. 4.1.3.18) that has a single amino acid change, i.e., W497L, that confers sulfonyl urea herbicide resistance (Int'l. App. Pub. No. WO 2006/052870). AHAS is the first common enzyme in the pathway for the biosynthesis of branched-chain amino acids, i.e., valine, leucine, isoleucine, and it is the target of the sulfonylurea and imidazolinone herbicides.

Fermentation Processes for Polyunsaturated Fatty Acid Production

[0198] The transformed host cell is grown under conditions that optimize expression of PUFA biosynthetic genes and produce the greatest and most economical yield of desired PUFAs. 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. Oleaginous yeast of interest, such as *Yarrowia lipolytica*, are generally grown in a complex medium such as yeast extract-peptone-dextrose broth (YPD) or a defined minimal media that lacks a component necessary for growth and forces selection of the desired expression cassettes (e.g., Yeast Nitrogen Base (DIFCO Laboratories, Detroit, Mich.)).

[0199] 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. Suitable sources of carbon encompass a wide variety of sources, with sugars, glycerol and/or fatty acids being preferred. Most preferred is glucose and/or fatty acids containing between 10-22 carbons.

[0200] 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 other components known to those skilled in the art suitable for the growth of the oleaginous yeast and the promotion of the enzymatic pathways of PUFA 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)).

[0201] Preferred growth media for the methods and host cells described herein are common commercially prepared media, such as Yeast Nitrogen Base (DIFCO Laboratories, Detroit, Mich.). Other defined or synthetic growth media may also be used and the appropriate medium for growth of the transformant host cells is well known in 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.

[0202] Typically, accumulation of increased amounts of PUFAs and TAGs 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 oils in oleaginous yeast. 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.

Purification and Processing of PUFA Oils

[0203] Fatty acids, including PUFAs, may be found in the host organisms as free fatty acids or in esterified forms such as acylglycerols, phospholipids, sulfolipids or glycolipids. These fatty acids may be extracted from the host cells through a variety of means well-known in the art. One review of extraction techniques, quality analysis and acceptability standards for yeast lipids is that of Z. Jacobs (*Critical Reviews in Biotechnology,* 12(5/6):463-491 (1992)). A brief review of downstream processing is also available by A. Singh and O. Ward (*Adv. Appl. Microbiol.,* 45:271-312 (1997)).

[0204] In general, means for the purification of fatty acids (including PUFAs) may include extraction (e.g., U.S. Pat. No. 6,797,303 and U.S. Pat. No. 5,648,564) with organic solvents, sonication, supercritical fluid extraction (e.g., using carbon dioxide), saponification and physical means such as presses, or combinations thereof. See U.S. Pat. No. 7,238,482.

Oils for Use in Foodstuffs, Health Food Products, Pharmaceuticals and Animal Feeds

[0205] The market place contains many food and feed products, incorporating ω-3 and/or ω-6 fatty acids, particularly ALA, GLA, ARA, EPA, DPA and DHA. It is contemplated that the microbial biomass comprising long-chain PUFAs, partially purified microbial biomass comprising PUFAs, purified microbial oil comprising PUFAs, and/or purified PUFAs made by the methods and host cells described herein impart health benefits, upon ingestion of foods or feed improved by their addition. These oils can be added to food analogs, drinks, meat products, cereal products, baked foods, snack foods and dairy products, to name a few. See U.S. Pat. App. Pub. No. 2006/0094092, hereby incorporated herein by reference.

[0206] These compositions may impart health benefits by being added to medical foods including medical nutritionals, dietary supplements, infant formula and pharmaceuticals. The skilled artisan will appreciate the amount of the oils to be added to food, feed, dietary supplements, nutriceuticals, pharmaceuticals, and other ingestible products as to impart health benefits. Health benefits from ingestion of these oils are described in the art, known to the skilled artisan and continuously investigated. Such an amount is referred to herein as an "effective" amount and depends on, among other things, the nature of the ingested products containing these oils and the physical conditions they are intended to address.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0207] As demonstrated in the Examples and summarized in Table 5, infra, disruptions in the C-terminal portion of the C_3HC_4 zinc ring finger motif of YlPex10p, deletion of the entire chromosomal YlPex10 gene or of the entire chromosomal YlPex16 gene, deletion of both the entire chromosomal YlPex10 and the YlPex16 gene, and deletion of the entire chromosomal YlPex3 gene all resulted in an engineered PUFA-producing strain of *Yarrowia lipolytica* that had an increased weight percent of PUFAs as a percent of total fatty acids, relative to the parental strain whose native Pex protein had no disruption. Expression of an extrachromosomal YlPex10p in an engineered EPA-producing strain of *Yarrowia lipolytica* that possessed a disruption in the genomic Pex10p and an increased amount of PUFAs in the total lipid fraction and in the oil fraction reversed the effect.

[0208] Table 5 compiles data from Examples 3, 4, 5, 7, 9, 11 and 12, such that trends concerning total lipid content ["TFAs % DCW"], concentration of a given fatty acid(s) expressed as a weight percent of total fatty acids ["% TFAs"], and content of a given fatty acid(s) as its percent of the dry cell weight ["% DCW"] can be deduced, based on the presence/absence of a Pex disruption or knockout. "Desired PUFA % TFAs" and "Desired PUFA % DCW" quantify the particular concentration or content, respectively, of the desired PUFA product (i.e., DGLA or EPA) that the engineered PUFA biosynthetic pathway was designed to produce. "All PUFAs" includes LA, ALA, EDA, DGLA, ETrA, ETA and EPA, while "C20 PUFAs" is limited to EDA, DGLA, ETrA, ETA and EPA.

TABLE 5

PUFA % TF As and % DCW In *Yarrowia lipolytica* Strains With Mutant Pex Genes

				% TF As			% DCW		
Example	Strain	Genomic Pex Gene	TF A % DCW	Desired PUFA	All PUFAs	C20 PUFAs	Desired PUFA	All PUFAs	C20 PUFAs
3, 4	Y4086	Wildtype Pex10	28.6	9.8 [EPA]	60.1	25.2	2.8 [EPA]	17.2	7.2
	Y4128	Mutant* Pex10	11.2	42.8 [EPA]	79.3	57.9	4.8 [EPA]	8.9	6.4
5	Y4128U1 + pFBAIn-PEX10	Mutant* Pex10 + Plasmid Wildtype Pex10 within chimeric FBAINm::Pex10::Pex20 gene	29.2	10.8 [EPA]	60	27.3	3.1 [EPA]	17.5	8.0
	Y4128U1 + pPEX10-1	Mutant* Pex10 + Plasmid Wildtype Pex10 within Pex10-5' (500 bp)::Pex10::Pex10-3' gene	27.1	10.7 [EPA]	60.1	26.7	2.9 [EPA]	16.2	7.2
	Y4128U1 + pPEX10-2	Mutant* Pex10 + Plasmid Wildtype Pex10 within Pex10-5' (991 bp)::Pex10::Pex10-3' gene	28.5	10.8 [EPA]	59	26.9	3.1 [EPA]	16.8	7.7
	Y4128U1 + control	Mutant* Pex10	22.8	27.7 [EPA]	62.6	42.3	6.3 [EPA]	14.2	9.6
7	Y4184U	Wildtype Pex10	11.8	20.6 [EPA]	nq◆	nq◆	2.4 [EPA]	nq◆	nq◆
			8.8	23.2 [EPA]	nq◆	nq◆	2.0 [EPA]	nq◆	nq◆
	Y4184U ΔPex10	Mutant Pex10	17.6	43.2 [EPA]	nq◆	nq◆	7.6 [EPA]	nq◆	nq◆
			13.2	46.1 [EPA]	nq◆	nq◆	6.1 [EPA]	nq◆	nq◆
9	Y4036 (avg)	Wildtype Pex16	Nq◆	23.4 [DGLA]	61.5	33.7	nq◆	nq◆	nq◆
	Y4036 (ΔPex16) (avg)	Mutant Pex16	Nq◆	43.4 [DGLA]	69.1	49.1	nq◆	nq◆	nq◆
11	Y4305U (Δpex10) (avg)	Mutant Pex10 and Wildtype Pex16	30	44.7 [EPA]	76.6	55.4	13.4 [EPA]	23.0	16.6
	Y4305 (ΔPex10, ΔPex16) (avg)	Mutant Pex10, Mutant Pex16	30	48.3 [EPA]	79.0	57.7	14.5 [EPA]	23.7	17.3
12	Y4036	Wildtype Pex3	4.7	19 [DGLA]	57	27	0.9 [DGLA]	2.7	1.3
	Y4036 (ΔPex3)	Mutant Pex3	6.1	46 [DGLA]	68	56	2.8 [DGLA]	4.4	3.4
			5.9	46 [DGLA]	68	56	2.7 [DGLA]	4.0	3.3

*Pex10 disruption in Y4128 results in a truncated protein, wherein the last 32 amino acids of the C-terminus (corresponding to the C-terminal portion of the C_3HC_4 zinc ring finger motif) are not present.
◆nq = not quantified

[0209] Although data cannot be directly compared between Examples, as a result of different *Yarrowia* strains and growth conditions, the following conclusions can be drawn (relative to the parental strain whose native Pex protein had not been disrupted or the parental strain that was expressing a "replacement" copy of the disrupted native Pex protein):

[0210] 1) Pex disruption in a PUFA-producing *Yarrowia* results in an increase in the weight percent of a single PUFA, for example EPA or DLGA, relative to the weight percent of total fatty acids (% TFAs) in the total lipid fraction and in the oil fraction;

[0211] 2) Pex disruption in a PUFA-producing *Yarrowia* results in an increase in the weight percent of C_{20} PUFAs relative to the weight percent of total fatty acids in the total lipid fraction and in the oil fraction;

[0212] 3) By the extension of point 1), Pex disruption in a PUFA-producing *Yarrowia* results in an increase in the amount of any and all combinations of PUFAs relative to

the weight percent of total fatty acids in the total lipid fraction and in the oil fraction; and

[0213] 4) Pex disruption in a PUFA-producing *Yarrowia* results in an increase in the percent of a single PUFA, for example EPA or DLGA, relative to the dry cell weight.

[0214] Variable results are observed when comparing the effects of Pex disruptions in "All PUFAs % DCW", "C20 PUFAs % DCW" and TFA % DCW. Specifically, in some cases, the Pex disruption in the PUFA-producing *Yarrowia* results in an increased amount of C_{20} PUFAs or All PUFAs, as a percent of dry cell weight, in the total lipid fraction and in the oil fraction (relative to the parental strain whose native Pex protein had not been disrupted). In other cases, there is a diminished amount of C_{20} PUFAs or All PUFAs, as a percent of dry cell weight, in the total lipid fraction and in the oil fraction (relative to the parental strain whose native Pex protein had not been disrupted). Similar results are observed with respect to the total lipid content (TFA % DCW), in that the effect of the Pex disruption can either result in an increase in total lipid content or a decrease.

[0215] Although each of the above generalizations are of interest, it is particularly useful to examine the effect of the Pex disruptions on the ratio of the desired PUFA which the organism was engineered to produce relative to the amount of total PUFAs.

[0216] For example, 54% of the PUFAs (as a % TFAs) were EPA in strain Y4128 containing the Pex10 disruption that resulted in truncation of the last 32 amino acids of the C-terminus, while only 16.3% of the PUFAs (as a % TFAs) were EPA in the parent strain, Y4086. Thus, the disruption was responsible for a 3.3-fold increase in the amount of the desired PUFA (as % TFAs) (Examples 3, 4). In a similar manner, 62.8% of the PUFAs (as a % TFAs) were DGLA in strain Y4036 (ΔPex16), while only 38.1% the PUFAs (as a % TFAs) were DGLA in Y4036—a 1.65 fold increase (Example 9). And, 67.7% of the PUFAs (as a % TFAs) were DGLA in strain Y4036 (ΔPex3), while only 33.3% the PUFAs (as a % TFAs) were DGLA in Y4036—a 2.0 fold increase (Example 12). These results support the hypothesis that the Pex disruption results in a selective increase in the amount, as a % TFAs, of the desired PUFA which the organism was engineered to produce in the total lipid and oil fractions.

[0217] Less significant selectivity is observed when examining the effect of Pex disruptions on the ratio of C20 PUFAs relative to the amount of total PUFAs. For example, 73% of the PUFAs (as a % TFAs) were C20 PUFAs in strain Y4128 containing the Pex10 disruption, while only 42% of the PUFAs (as a % TFAs) were C20 PUFAs in strain Y4086. Thus, the disruption was responsible for a 1.7-fold increase in the amount of C20 PUFAs that accumulated in the total lipid and oil fractions, relative to the total PUFAs (Examples 3, 4). In a similar manner, 71% of the PUFAs (as a % TFAs) were C20 PUFAs in strain Y4036 (ΔPex16), while only 54.8% the PUFAs (as a % TFAs) were C20 PUFAs in Y4036—a 1.3 fold increase (Example 9). And, 82.4% of the PUFAs (as a % TFAS) were C20 PUFAs in strain Y4036 (ΔPex3), while only 47.4% the PUFAs (as a % TFAs) were C20 PUFAs in Y4036—a 1.7 fold increase (Example 12).

[0218] On the basis of the teachings and results described herein, it is expected that the feasibility and commercial utility of utilizing various disruptions in native genes encoding peroxisome biogenesis factor proteins as a means to increase the amount of PUFAs produced in a PUFA-producing eukaryotic organism will be appreciated. The PUFA-producing eukaryotic organism can synthesize a variety of ω-3 and/or ω-6 PUFAs, using either the Δ9 elongase/Δ8 desaturase pathway or the Δ6 desaturase/Δ6 elongase pathway.

EXAMPLES

[0219] 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

[0220] 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, N.Y. (1989) (Maniatis); 2) T. J. Silhavy, M. L. Bennan, and L. W. Enquist, Experiments with Gene Fusions; Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y. (1984); and, 3) Ausubel, F. M. et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience, Hoboken, N.J. (1987).

[0221] 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, Mass. (1989). All reagents, restriction enzymes and materials used for the growth and maintenance of microbial cells were obtained from Aldrich Chemicals (Milwaukee, Wis.), DIFCO Laboratories (Detroit, Mich.), New England Biolabs, Inc. (Beverly, Mass.), 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.

[0222] General molecular cloning was performed according to standard methods (Sambrook et al., supra). DNA sequence was generated on an ABI Automatic sequencer using dye terminator technology (U.S. Pat. No. 5,366,860; EP 272,007) using a combination of vector and insert-specific primers. Sequence editing was performed in Sequencher (Gene Codes Corporation, Ann Arbor, Mich.). All sequences represent coverage at least two times in both directions. Unless otherwise indicated herein comparisons of genetic sequences were accomplished using DNASTAR software (DNASTAR Inc., Madison, Wis.).

[0223] The meaning of abbreviations is as follows: "sec" means second(s), "min" means minute(s), "h" means hour(s), "d" means day(s), "μL" means microliter(s), "mL" means milliliter(s), "L" means liter(s), "μM" means micromolar, "mM" means millimolar, "M" means molar, "mmol" means millimole(s), "pmole" mean micromole(s), "g" means gram (s), "μg" means microgram(s), "ng" means nanogram(s), "U" means unit(s), "bp" means base pair(s) and "kB" means kilobase(s).

Nomenclature for Expression Cassettes:

[0224] The structure of an expression cassette is represented by a simple notation system of "X::Y::Z", wherein X

describes the promoter fragment, Y describes the gene fragment, and Z describes the terminator fragment, which are all operably linked to one another.

Transformation and Cultivation of *Yarrowia lipolytica*

[0225] *Yarrowia lipolytica* strain ATCC #20362 was purchased from the American Type Culture Collection (Rockville, Md.). *Yarrowia lipolytica* strains were routinely grown at 28-30° C. in several media, according to the recipes shown below. Agar plates were prepared as required by addition of 20 g/L agar to each liquid media, according to standard methodology.

[0226] YPD agar medium (per liter): 10 g of yeast extract [Difco], 20 g of Bacto peptone [Difco], and 20 g of glucose.

[0227] Basic Minimal Media (MM) (per liter): 20 g glucose, 1.7 g yeast nitrogen base without amino acids, 1.0 g proline, and pH 6.1 (not adjusted).

[0228] Minimal Media+Uracil (MM+uracil or MMU) (per liter): Prepare MM media as above and add 0.1 g uracil and 0.1 g uridine.

[0229] Minimal Media+Uracil+Sulfonylurea (MMU+SU) (per liter): Prepare MMU media as above and add 280 mg sulfonylurea.

[0230] Minimal Media+Leucine+Lysine (MMLeuLys) (per liter): Prepare MM media as above and add 0.1 g leucine and 0.1 g lysine.

[0231] Minimal Media+5-Fluoroorotic Acid (MM+5-FOA) (per liter): 20 g glucose, 6.7 g Yeast Nitrogen base, 75 mg uracil, 75 mg uridine and appropriate amount of FOA (Zymo Research Corp., Orange, Calif.), based on FOA activity testing against a range of concentrations from 100 mg/L to 1000 mg/L (since variation occurs within each batch received from the supplier).

[0232] High Glucose Media (HGM) (per liter): 80 glucose, 2.58 g KH_2PO_4 and 5.36 g K_2HPO_4, pH 7.5 (do not need to adjust).

[0233] Fermentation medium without Yeast Extract (FM without YE) (per liter): 6.70 g Yeast Nitrogen base, 6.00 g KH_2PO_4, 2.00 g K_2HPO_4, 1.50 g $MgSO_4*7H_2O$ and 20 g Glucose.

[0234] Fermentation medium (FM) (per liter): Prepare FM without YE media as above and add 5.00 g Yeast extract (BBL).

[0235] Synthetic Dextrose Media (SD) (per liter): 6.7 g Yeast Nitrogen base with ammonium sulfate and without amino acids; and 20 g glucose.

[0236] Complete Minimal Glucose Broth Minus Uracil (CSM-Ura): Catalog No. C8140, Teknova, Hollister, Calif. (0.13% amino acid dropout powder minus uracil. 0.17% yeast nitrogen base, 0.5% $(NH_4)_2SO_4$, 2.0% glucose).

[0237] Transformation of *Y. lipolytica* was performed according to the method of Chen, D. C. et al. (*Appl. Microbiol. Biotechnol.,* 48(2):232-235 (1997)), unless otherwise noted. Briefly, *Yarrowia* was streaked onto a YPD plate and grown at 30° C. for approximately 18 hr. Several large loopfuls of cells were scraped from the plate and resuspended in 1 mL of transformation buffer containing: 2.25 mL of 50% PEG, average MW 3350; 0.125 mL of 2 M Li acetate, pH 6.0; and 0.125 mL of 2 M DTT. Then, approximately 500 ng of linearized plasmid DNA was incubated in 100 μl of resuspended cells, and maintained at 39° C. for 1 hr with vortex mixing at 15 min intervals. The cells were plated onto selection media plates and maintained at 30° C. for 2 to 3 days.

Fatty Acid Analysis Of *Yarrowia lipolytica*

[0238] For fatty acid analysis, cells were collected by centrifugation and lipids were extracted as described in Bligh, E. G. & Dyer, W. J. (*Can. J. Biochem. Physiol.,* 37:911-917 (1959)). Fatty acid methyl esters ["FAMEs"] were prepared by transesterification of the lipid extract with sodium methoxide (Roughan, G., and Nishida I., *Arch Biochem Biophys.,* 276(1):3846 (1990)) and subsequently analyzed with a Hewlett-Packard 6890 GC fitted with a 30-m×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.

[0239] For direct base transesterification, *Yarrowia* culture (3 mL) was harvested, washed once in distilled water, and dried under vacuum in a Speed-Vac for 5-10 min. Sodium methoxide (100 μl of 1%) was added to the sample, and then the sample was vortexed and rocked for 20 min. 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 as described above.

Example 1

Generation of *Yarrowia lipolytica* Strain Y4086 to Produce about 14% EPA of Total Lipids Via the Δ9 Elongase/Δ8 Desaturase Pathway

[0240] The present Example describes the construction of strain Y4086, derived from *Yarrowia lipolytica* ATCC #20362, capable of producing about 14% EPA relative to the total lipids via expression of a Δ9 elongase/Δ8 desaturase pathway (FIG. **3**A).

[0241] The development of strain Y4086 required the construction of strain Y2224 (a FOA resistant mutant from an autonomous mutation of the Ura3 gene of wildtype *Yarrowia* strain ATCC #20362), strain Y4001 (producing 17% EDA with a Leu− phenotype), strain Y4001U (Leu− and Ura− phenotype), strain Y4036 (producing 18% DGLA with a Leu− phenotype), strain Y4036U (Leu− and Ura− phenotype) and strain Y4070 (producing 12% ARA with a Ura− phenotype). Further details regarding the construction of strains Y2224, Y4001, Y4001U, Y4036, Y4036U and Y4070 are described in Example 7 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference.

[0242] The final genotype of strain Y4070 with respect to wildtype *Yarrowia lipolytica* ATCC #20362 was Ura3-, unknown 1-, unknown 3-, Leu+, Lys+, GPD::FmD12::Pex20, YAT1::FmD12::OCT, YAT1::ME3S::Pex16, GPAT::EgD9e::Lip2, EXP1::EgD9eS::Lip1, FBAINm::EgD9eS::Lip2, FBAINm::EgD8M::Pex20, EXP1::EgD8M::Pex16, FBAIN::EgD5::Aco, EXP1::EgD5S::Pex20, YAT1::RD5S::OCT (wherein FmD12 is a *Fusarium moniliforme* Δ12 desaturase gene [Int'l. App. Pub. No. WO 2005/047485]; ME3S is a codon-optimized $C_{16/18}$ elongase gene, derived from *Mortierella alpina* [Int'l. App. Pub. No. WO 2007/046817]; EgD9e is a *Euglena gracilis* Δ9 elongase gene [Int'l. App. Pub. No. WO 2007/061742]; EgD9eS is a codon-optimized Δ9 elongase gene, derived from *Euglena gracilis* [Int'l. App. Pub. No. WO 2007/061742]; EgD8M is a synthetic mutant Δ8 desaturase [Int'l. App. Pub. No. WO 2008/073271], derived from *Euglena gracilis* [U.S. Pat. No. 7,256,033]; EgD5 is a *Euglena gracilis* Δ5 desaturase [U.S. Pat. App. Pub. US 2007-0292924-A1]; EgD5S is a codon-optimized Δ5 desaturase gene, derived from *Euglena gracilis* [U.S. Pat. App. Pub. No. 2007-0292924]; and RD5S is a

codon-optimized Δ5 desaturase, derived from *Peridinium* sp. CCMP626 [U.S. Pat. App. Pub. No. 2007-0271632]).

Generation of Y4086 Strain to Produce about 14% EPA of Total Lipids

[0243] Construct pZP3-Pa777U (FIG. **3**B; SEQ ID NO:28), described in Table 19 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference, was generated to integrate three Δ17 desaturase genes into the Pox3 loci (GenBank Accession No. AJ001301) of strain Y4070, to thereby enable production of EPA. The Δ17 desaturase genes were PaD17, a *Pythium aphanidermatum* Δ17 desaturase (Int'l. App. Pub. No. WO 2008/054565), and PaD17S, a codon-optimized Δ17 desaturase derived from *Pythium aphanidermatum* (Int'l. App. Pub. No. WO 2008/054565).

[0244] The pZP3-Pa777U plasmid was digested with AscI/SphI, and then used for transformation of strain Y4070 according to the General Methods. The transformant cells were plated onto MM plates and maintained at 30° C. for 2 to 3 days. Single colonies were re-streaked onto MM plates, and then inoculated into liquid MMLeuLys at 30° C. and shaken at 250 rpm/min for 2 days. The cells were collected by centrifugation, lipids were extracted, and FAMEs were prepared by trans-esterification, and subsequently analyzed with a Hewlett-Packard 6890 GC.

[0245] GC analyses showed the presence of EPA in the transformants containing the 3 chimeric genes of pZP3-Pa777U, but not in the parent Y4070 strain. Most of the selected 96 strains produced 10-13% EPA of total lipids. There were 2 strains (i.e., #58 and #79) that produced about 14.2% and 13.8% EPA of total lipids. These two strains were designated as Y4085 and Y4086, respectively.

[0246] The final genotype of strain Y4086 with respect to wildtype *Yarrowia lipolytica* ATCC #20362 was Ura3+, Leu+, Lys+, unknown 1-, unknown 2-, YALI0F24167g-, GPD::FmD12::Pex20, YAT1::FmD12::OCT, YAT1::ME3S::Pex16, GPAT::EgD9e::Lip2, EXP1::EgD9eS::Lip1, FBAINm::EgD9eS::Lip2, FBAINm::EgD8M::Pex20, EXP1::EgD8M::Pex16, FBAIN::EgD5::Aco, EXP1::EgD5S::Pex20, YAT1::RD5S::OCT, YAT1::PaD17S::Lip1, EXP1::PaD17::Pex16, FBAINm::PaD17::Aco.

Example 2

Generation of *Yarrowia* Lipolytica Strain Y4128 to Produce about 37% EPA of Total Lipids Via the Δ9 Elongase/Δ8 Desaturase Pathway

[0247] The present Example describes the construction of strain Y4128, derived from *Yarrowia lipolytica* ATCC #20362, capable of producing about 37.6% EPA relative to the total lipids (i.e., greater than a 2-fold increase in EPA concentration as percent of total fatty acids with respect to Y4086; FIG. **3**A).

[0248] The development of strain Y4128 required the construction of strains Y2224, Y4001, Y4001U, Y4036, Y4036U, Y4070 and Y4086 (described in Example 1), as well as construction of strain Y4086U1 (Ura−).

Generation Of Strain Y4086U1 (Ura−)

[0249] Strain Y4086U1 was created via temporary expression of the Cre recombinase enzyme in construct pY117 (FIG. **4**A; SEQ ID NO:29; described in Table 20 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) within strain Y4086 to produce a Ura− phenotype. This released the LoxP sandwiched Ura3 gene from the genome. The mutated *Yarrowia* acetohydroxyacid synthase ["AHAS"; E.C. 4.1.3.18] enzyme (i.e., GenBank Accession No. XP_501277, comprising a W497L mutation as set forth in SEQ ID NO:27; see Int'l. App. Pub. No. WO 2006/052870) in plasmid pY117 conferred sulfonyl urea herbicide resistance (SU^R), which was used as a positive screening marker.

[0250] Plasmid pY117 was used to transform strain Y4086 according to the General Methods. Following transformation, the cells were plated onto MMU+SU (280 μg/mL sulfonylurea; also known as chlorimuron ethyl, E. I. duPont de Nemours & Co., Inc., Wilmington, Del.) plates and maintained at 30° C. for 2 to 3 days. The individual SU^R colonies grown on MMU+SU plates were picked, and streaked into YPD liquid media at 30° C. and shaken at 250 rpm/min for 1 day to cure the pY117 plasmid. The grown cultures were streaked onto MMU plates. After two days at 30° C., the individual colonies were re-streaked onto MM and MMU plates. Those colonies that could grow on MMU, but not on MM plates were selected. Two of these strains with Ura-phenotypes were designated as Y4086U1 and Y4086U2.

Generation of Y4128 Strain to Produce about 37% EPA of Total Lipids

[0251] Construct pZP2-2988 (FIG. **4**B; SEQ ID NO:30; described in Table 21 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) was generated to integrate one Δ12 desaturase gene (i.e., FmD12S, a codon-optimized Δ12 desaturase gene derived from *Fusarium moniliforme* [Int'l. App. Pub. No. WO 2005/047485]), two Δ8 desaturase genes (i.e., EgD8M) and one Δ9 elongase gene (i.e., EgD9eS) into the Pox2 loci (GenBank Accession No. AJ001300) of strain Y4086U1, to thereby enable higher level production of EPA. The pZP2-2988 plasmid was digested with AscI/SphI, and then used for transformation of strain Y4086U1 according to the General Methods. The transformant cells were plated onto MM plates and maintained at 30° C. for 2 to 3 days. Single colonies were re-streaked onto MM plates, and then inoculated into liquid MMLeuLys at 30° C. and shaken at 250 rpm/min for 2 days. The cells were collected by centrifugation, resuspended in HGM and then shaken at 250 rpm/min for 5 days. The cells were collected by centrifugation, lipids were extracted, and FAMEs were prepared by trans-esterification, and subsequently analyzed with a Hewlett-Packard 6890 GC.

[0252] GC analyses showed that most of the selected 96 strains produced 12-15.6% EPA of total lipids. There were 2 strains (i.e., #37 within Group I and #33 within Group II) that produced about 37.6% and 16.3% EPA of total lipids. These two strains were designated as Y4128 and Y4129, respectively.

[0253] The final genotype of strain Y4128 with respect to wildtype *Yarrowia lipolytica* ATCC #20362 was: YALI0F24167g-, Pex10-, unknown 1-, unknown 2-, GPD::FmD12::Pex20, YAT1::FmD12::OCT, GPM/FBAIN::FmD12S::OCT, YAT1::ME3S::Pex16, GPAT::EgD9e::Lip2, EXP1::EgD9eS::Lip1, FBAINm::EgD9eS::Lip2, FBA::EgD9eS::Pex20, FBAINm::EgD8M::Pex20, EXP1::EgD8M::Pex16, GPDIN::EgD8M::Lip1, YAT1::EgD8M::Aco, FBAIN::EgD5::Aco, EXP1::EgD5S::Pex20, YAT1::RD5S::OCT, YAT1::PaD17S::Lip1, EXP1.::PaD17::Pex16, FBAINm::PaD17::Aco.

[0254] *Yarrowia lipolytica* strain Y4128 was deposited with the American Type Culture Collection on Aug. 23, 2007 and bears the designation ATCC PTA-8614.

Generation of Y4128U Strains With A Ura− Phenotype

[0255] In order to disrupt the Ura3 gene in strain Y4128, construct pZKUE3S (FIG. **5**A; SEQ ID NO:31; described in Table 22 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) was created to integrate a EXP1::ME3S::Pex20 chimeric gene into the Ura3 gene of strain Y4128. Plasmid pZKUE3S was digested with SphI/PacI, and then used to transform strain Y4128 according to the General Methods. Following transformation, cells were plated onto MM+5-FOA selection plates and maintained at 30° C. for 2 to 3 days.

[0256] A total of 24 transformants grown on MM+5-FOA selection plates were picked and re-streaked onto fresh MM+5-FOA plates. The cells were stripped from the plates, lipids were extracted, and FAMEs were prepared by trans-esterification, and subsequently analyzed with a Hewlett-Packard 6890 GC.

[0257] GC analyses showed the presence of between 10-15% EPA in all of the transformants with pZKUE3S from plates. The strains identified as #3, #4, #10, #12, #19 and #21 that produced 12.9%, 14.4%, 15.2%, 15.4%, 14% and 10.9% EPA of total lipids were designated as Y4128U1, Y4128U2, Y4128U3, Y4128U4, Y4128U5 and Y4128U6, respectively (collectively, Y4128U).

[0258] The discrepancy in the % EPA quantified in Y4128 (37.6%) versus Y4128U (average 13.8%) is based on differing growth conditions. Specifically, the former culture was analyzed following two days of growth in liquid culture, while the latter culture was analyzed after growth on an agar plate. The Applicants have observed a 2-3 fold increase in % EPA, when comparing results from agar plates to those in liquid culture. Thus, although results are not directly comparable, both Y4128 and Y4128U strains demonstrate production of EPA.

Example 3

Determination of Total Lipid Content of *Yarrowia lipolytica* Strain Y4128

[0259] The total amount of lipid produced by strain Y4128 and the percentage of each fatty acid species in the lipid were measured by GC analysis. Specifically, total lipids were extracted, and FAMEs were prepared by trans-esterification, and subsequently analyzed with a Hewlett-Packard 6890 GC, as described in the General Methods.

[0260] Dry cell weight was determined by collecting cells from 10 mL of culture via centrifugation, washing the cells with water once to remove residual medium, drying the cells in a vacuum oven at 80° C. overnight, and weighing the dried cells. The total amount of FAMEs in a sample was determined by comparing the areas of all peaks in the GC profile with the peak area of an added known amount of internal standard C15:0 fatty acid.

[0261] Based on the above analyses, lipid content as a percentage of dry cell weight (DCW) and lipid composition was determined for strains Y4086 and Y4128. Strain Y4128 had decreased lipid content with respect to strain Y4086 (11.2 TFAs % DCW versus 28.6 TFAs % DCW). In contrast, strain Y4128 had elevated EPA concentrations among lipids with respect to strain Y4086, as shown below in Table 6. Fatty acids are identified as 18:0 (stearic acid), 18:1 (oleic acid), LA, ALA, EDA, DGLA, ETrA, ETA and EPA; fatty acid compositions were expressed as the weight percent (wt. %) of total fatty acids (TFAs).

TABLE 6

Lipid Composition in *Yarrowia lipolytica* Strains Y4086 And Y4128

Sample	18:0	18:1	18:2 [LA]	18:3 (n-3) [ALA]	20:2 [EDA]	20:3 (n-6) [DGLA]	20:3 (n-3) [ETrA]	20:4 (n-3) [ETA]	20:5 (n-3) [EPA]
Y4086	4.6	26.8	28.0	6.9	7.6	0.9	4.9	2.0	9.8
Y4128	1.8	6.7	19.6	1.8	4.2	3.4	1.5	6.0	42.8

EPA content in the cell, expressed as mg EPA/g dry cell and calculated according to the following formula: (% of EPA/Lipid) * (% of Lipid/dry cell weight) * 0.1, increased from 28 mg EPA/g DCW in strain Y4086 to 47.9 mg EPA/g DCW in strain Y4128.

[0262] Thus, the results in Table 6 showed that compared to the parent strain Y4086, strain Y4128 had a lower total lipid content (TFAs % DCW) (11.2% versus 28.6%), higher EPA % TFAs (42.8% versus 9.8%), and higher EPA % DCW (4.8% versus 2.8%). Additionally, strain Y4128 had a 3.3-fold increase in the amount of EPA relative to the total PUFAs (54% of the PUFAs [as a % TFAs] versus 16.3% of the PUFAs [as a % TFAs]) and a 1.7-fold increase in the amount of C20 PUFAs relative to the total PUFAs (73% of the PUFAs [as a % TFAs] versus 42% of the PUFAs [as a % TFAs]).

Example 4

Determination of the Integration Site of pZP2-2988 in *Yarrowia lipolytica* Strain Y4128 as a Pex10Integration

[0263] The genomic integration site of pZP2-2988 in strain Y4128 was determined by genome walking using the Universal GenomeWalker™ Kit from Clontech (Palo Alto, Calif.), following the manufacturer's recommended protocol. Based on the sequence of the plasmid, the following primers were designed for genome walking: pZP-GW-5-1 (SEQ ID NO:32), pZP-GW-5-2 (SEQ ID NO:33), pZP-GW-5-3 (SEQ ID NO:34), pZP-GW-54 (SEQ ID NO:35), pZP-GW-3-1 (SEQ ID NO:36), pZP-GW-3-2 (SEQ ID NO:37), pZP-GW-3-3 (SEQ ID NO:38) and pZP-GW-34 (SEQ ID NO:39).

[0264] Genomic DNA was prepared from strain Y4128 using the Qiagen Miniprep kit with a modified protocol. Cells were scraped off a YPD medium plate into a 1.5 mL microfuge tube. Cell pellet (100 μl) was resuspended with 250 μl of buffer P1 containing 0.125 M β-mercaptoethanol

and 1 mg/mL zymolyase 20 T (MP Biomedicals, Inc., Solon, Ohio). The cell suspension was incubated at 37° C. for 30 min. Buffer P2 (250 μl) was then added to the tube. After mixing by inverting the tube for several times, 350 μl of buffer N3 was added. The mixture was then centrifuged at 14,000 rpm for 5 min in a microfuge. Supernatant was poured into a Qiagen miniprep spin column, and centrifuged for 1 min. The column was washed once by adding 0.75 mL of buffer PE, followed by centrifugation at 14,000 rpm for 1 min. The column was dried by further centrifugation at 14,000 rpm for 1 min. Genomic DNA was eluted by adding 50 μl of buffer EB to the column, allowed to sit for 1 min and centrifuged at 14,000 rpm for 1 min.

[0265] Purified genomic DNA was used for genome walking. The DNA was digested with restriction enzymes DraI, EcoRV, PvuII and StuI separately, according to the protocol of the GenomeWalker kit. For each digestion, the reaction mixture contained 10 μl of 10× restriction buffer, 10 μl of the appropriate restriction enzyme and 8 μg of genomic DNA in a total volume of 100 μl. The reaction mixtures were incubated at 37° C. for 4 hrs. The digested DNA samples were then purified using Qiagen PCR purification kit following the manufacturer's protocol exactly. DNA samples were eluted in 16 μl water. Purified, digested genomic DNA samples were then ligated to the genome walker adaptor (infra). Each ligation mixture contained 1.9 μl of the genome walker adaptor, 1.6 μl of 10× ligation buffer, 0.5 μl T4 DNA ligase and 4 μl of the digested DNA. The reaction mixtures were incubated at 16° C. overnight. Then, 72 μl of 50 mM Tris HCl, 1 mM EDTA, pH 7.5 were added to each ligation mixture.

[0266] For 5'-end genome walking, four PCR reactions were carried out using 1 μl of each ligation mixture individually as template. In addition, each reaction mixture contained 1 μl of 10 μM primer pZP-GW-5-1 (SEQ ID NO:32), 1 μl of 10 μM kit-supplied Genome Walker adaptor, 41 μl water, 5 μl 10× cDNA PCR reaction buffer and 1 μl Advantage cDNA polymerase mix from Clontech. The sequence of the Genome Walker adaptor (SEQ ID NOs:40 [top strand] and 41 [bottom strand]), is shown below:

```
5'-GTAATACGACTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGG
T-3'

3'-H2N-CCCGACCA-5'
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The PCR conditions were as follows: 95° C. for 1 min, followed by 30 cycles at 95° C. for 20 sec and 68° C. for 3 min, followed by a final extension at 68° C. for 7 min. The PCR products were each diluted 1:100 and 1 μl of the diluted PCR product used as template for a second round of PCR. The conditions were exactly the same except that pZP-GW-5-2 (SEQ ID NO:33) replaced pZP-GW-5-1 (SEQ ID NO:32).

[0267] For 3'-end genome walking, four PCR reactions were carried out as above, except primer pZP-GW-3-1 (SEQ ID NO:36) and nested adaptor primer (SEQ ID NO:42) were used. The PCR products were similarly diluted and used as template for a second round of PCR, using pZP-GW-3-2 (SEQ ID NO:37) to replace pZP-GW-3-1 (SEQ ID NO:36).

[0268] PCR products were analyzed by gel electrophoresis. One reaction product, using EcoRV digested genomic DNA as template and the primers pZP-GW-3-2 and nested adaptor primer, generated a ~1.6 kB fragment. This fragment was isolated, purified with a Qiagen gel purification kit and cloned into pCR2.1—TOPO. Sequence analysis showed that the fragment included both part of plasmid pZP2-2988 and the *Yarrowia* genomic DNA from chromosome C. The junction between them was at nucleotide position 139826 of chromosome C. This was inside the coding region of the Pex10 gene (GenBank Accession No. CAG81606; SEQ ID NO:10).

[0269] To determine the 5' end of the junction, PCR amplification was performed using genomic DNA from strain Y4128 as the template and primers Per10 F1 (SEQ ID NO:43) and ZPGW-5-5 (SEQ ID NO:44). The reaction mixture included 1 μl each of 20 μM primer, 1 μl genomic DNA, 22 μl water and 25 μl TaKaRa ExTaq 2× premix (TaKaRa Bio Inc., Otsu Shiga, Japan). The thermocycler conditions were: 94° C. for 1 min, followed by 30 cycles of 94° C. for 20 sec, 55° C. for 20 sec and 72° C. for 2 min, followed by a final extension at 72° C. for 7 min. A 1.6 kB DNA fragment was amplified and cloned into pCR2.1—TOPO. Sequence analysis showed that it was a chimeric fragment between *Yarrowia* genomic DNA from chromosome C and pZP2-2988. The junction was at nucleotide position 139817 of chromosome C. Thus, a 10 nucleotide segment of chromosome C was replaced by the AscI/SphI fragment from pZP2-2988 (FIG. **4**B) in strain Y4128. As a result, Pex10 in strain Y4128 was lacking the last 32 amino acids of the encoded protein.

[0270] Based on the above conclusions, the Y4128U strains isolated in Example 2 (supra) are referred to subsequently as Δpex10 strains. For clarity, strain Y4128U1 is equivalent to strain Y4128U1 (Δpex10).

Example 5

Plasmid Expression of Pex10In *Yarrowia lipolytica* Strain Y4128U1 (Δpex10)

[0271] Three plasmids that carried the *Y. lipolytica* Pex10 gene were constructed: 1) pFBAIn-PEX10 allowed the expression of the Pex100RF under the control of the FBAINm promoter; and, 2) pPEX10-1 and pPEX10-2 allowed the expression of Pex10 under control of the native Pex10 promoter, although pPEX10-1 used a shorter version (~500 bp) while pPEX10-2 used a longer version (~900 bp) of the promoter. Following construction of these expression plasmids and transformation, the effect of Pex10 plasmid expression on total oil and on EPA level in the *Y. lipolytica* strain Y4128U1 (Δpex10) was determined. Deletion of Pex10 resulted in an increased amount of EPA as a percent of TFAs, but a reduced amount of total lipid, as a percent of DCW, in the cell.

Construction of pFBAIn-PEX10, pPEX10-1 and pPEX10-2

[0272] To construct pFBAIn-PEX10, the primers Per10 F1 (SEQ ID NO:43) and Pe10 R (SEQ ID NO:45) were used to amplify the coding region of the Pex10 gene using *Y. lipolytica* genomic DNA as template. The PCR reaction mixture contained 1 μl each of 20 μM primers, 1 μl of *Y. lipolytica* genomic DNA (~100 ng), 25 μl ExTaq 2× premix and 22 μl water. The reaction was carried out as follows: 94° C. for 1 min, followed by 30 cycles of 94° C. for 20 sec, 55° C. for 20 sec and 72° C. for 90 sec, followed by a final extension of 72° C. for 7 min. The PCR product, a 1168 bp DNA fragment, was purified with a Qiagen PCR purification kit, digested with NcoI and NotI, and cloned into pFBAIn-MOD-1 (SEQ ID NO:46; FIG. **5**B) digested with the same two restriction enzymes.

[0273] Of the 8 individual clones subjected to sequence analysis, 2 had the correct sequence of Pex10 with no errors.

The components of pFBAIn-PEX10 (SEQ ID NO:47; FIG. **6**A) are listed below in Table 7.

TABLE 7

Components Of Plasmid pFBAIn-PEX10 (SEQ ID NO: 47)

RE Sites And Nucleotides Within SEQ ID NO: 47	Description Of Fragment And Chimeric Gene Components
BglII-BsiWI (6040-318)	FBAINm::Pex10::Pex20, comprising: FBAINm: *Yarrowia lipolytica* FBAINm promoter (U.S. Pat. No. 7,202,356); Pex10: *Y. lipolytica* Pex10 ORF (GenBank Accession No. AB036770, nucleotides 1038-2171; SEQ ID NO: 21); Pex20: Pex20 terminator sequence from *Yarrowia* Pex20 gene (GenBank Accession No. AF054613)
PacI-BglII (4530-6040)	*Yarrowia* URA3 (GenBank Accession No. AJ306421)
(3123-4487)	*Yarrowia* autonomous replicating sequence 18 (ARS18; GenBank Accession No. A17608)
(2464-2864)	*E. coli* f1 origin of replication
(1424-2284)	Ampicillin-resistance gene (Amp^R) for selection in *E. coli*
(474-1354)	ColE1 plasmid origin of replication

[0274] To construct pPEX10-1 and pPEX10-2, primers PEX10-R-BsiWI (SEQ ID NO:48), PEX10-F1-SalI (SEQ ID NO:49) and PEX10-F2-SalI (SEQ ID NO:50) were designed and synthesized. PCR amplification using genomic *Yarrowia lipolytica* DNA and primers PEX10-R-BsiWI and PEX10-F1-SalI generated a 1873 bp fragment containing the Pex100RF, 500 bp of the 5' upstream region and 215 bp of the 3' downstream region of the Pex10 gene, flanked by SalI and Bs/WI restriction sites at either end. This fragment was purified with the Qiagen PCR purification kit, digested with SalI and BsiWI, and cloned into pEXP-MOD-1 (SEQ ID NO:51; FIG. **6**B) digested with the same two enzymes to generate pPEX10-1 (SEQ ID NO:52; FIG. **7**A). Plasmid pEXP-MOD1 is similar to pFBAIn-MOD-1 (SEQ ID NO:46; FIG. **5**B) except that the FBAINm promoter in the latter was replaced with the EXP1 promoter. Table 8 lists the components of pPEX10-1.

TABLE 8

Components Of Plasmid pPEX10-1 (SEQ ID NO: 52)

RE Sites And Nucleotides Within SEQ ID NO: 52	Description Of Fragment And Chimeric Gene Components
SalI-BsiWI (5705-1)	Pex10-5'::Pex10::Pex10-3', comprising: Pex10-5': 500 bp of the 5' promoter region of *Yarrowia lipolytica* Pex10 gene;

TABLE 8-continued

Components Of Plasmid pPEX10-1 (SEQ ID NO: 52)

RE Sites And Nucleotides Within SEQ ID NO: 52	Description Of Fragment And Chimeric Gene Components
	Pex10: *Yarrowia lipolytica* Pex10 ORF (GenBank Accession No. AB036770, nucleotides 1038-2171; SEQ ID NO: 21); Pex10-3': 215 bp of Pex10 terminator sequence from *Yarrowia* Pex10 gene (GenBank Accession No. AB036770) [Note the entire Pex10-5'::Pex10::Pex10-3' expression cassette is labeled collectively as "PEX10" in the Figure]
PacI-SalI (4216-5703)	*Yarrowia* URA3 gene (GenBank Accession No. AJ306421)
(2806-4170)	*Yarrowia* autonomous replicating sequence 18 (ARS18; GenBank Accession No. A17608)
(2147-2547)	*E. coli* f1 origin of replication
(1107-1967)	Ampicillin-resistance gene (Amp^R) for selection in *E. coli*
(157-1037)	ColE1 plasmid origin of replication

[0275] PCR amplification of *Yarrowia lipolytica* genomic DNA using PEX10-R-BsiWI (SEQ ID NO:48) and PEX10-F2-SalI (SEQ ID NO:50) generated a 2365 bp fragment containing the PEX10 ORF, 991 bp of the 5' upstream region and 215 bp of the 3' downstream region of the Pex10 gene, flanked by SalI and BsiWI restriction sites at either end. This fragment was purified with a Qiagen PCR purification kit, digested with SalI and BsiWI, and cloned into similarly digested pEXP-MOD-1. This resulted in synthesis of pPEX10-2 (SEQ ID NO:53), whose construction is analogous to that of plasmid pPEX10-1 (Table 8, supra), with the exception of the longer Pex10-5' promoter in the chimeric Pex10-5'::Pex10::Pex10-3'gene.

Expression of Pex10 in Strain Y4128U1 (Δpex10)

[0276] Plasmids pFBAIN-MOD-1 (control; SEQ ID NO:46), pFBAIn-PEX10 (SEQ ID NO:47), pPEX10-1 (SEQ ID NO:52) and pPEX10-2 (SEQ ID NO:53) were transformed into Y4128U1 (Δpex10) according to the protocol in the General Methods. Transformants were plated on MM plates. The total lipid content and fatty acid composition of transformants carrying the above plasmids were analyzed as described in Example 3.

[0277] Lipid content as a percentage of dry cell weight (DCW) and lipid composition are shown below in Table 9. Specifically, fatty acids are identified as 18:0 (stearic acid), 18:1 (oleic acid), LA, ALA, EDA, DGLA, ETrA, ETA and EPA; fatty acid compositions were expressed as the weight percent (wt. %) of total fatty acids.

TABLE 9

Lipid Composition in *Yarrowia lipolytica* Strain Y4128U1 (Δpex10) Transformed With Various Pex10 Plasmids

Plasmid	TFA % DCW	18:0	18:1	18:2 [LA]	18:3 (ω3) [ALA]	20:2 [EDA]	20:3 (ω6) [DGLA]	20:3 (ω3) [ETrA]	20:4 (ω3) [ETA]	20:5 (ω3) [EPA]
pFBAIN-MOD-1	22.8	1.9	9.6	18.3	2.0	4.3	2.3	2.1	5.9	27.7
pFBAIN-PEX10	29.2	4.0	24.9	25.1	7.6	6.6	1.0	5.3	3.6	10.8

TABLE 9-continued

Lipid Composition in *Yarrowia lipolytica* Strain Y4128U1 (Δpex10) Transformed With Various Pex10 Plasmids										
Plasmid	TFA % DCW	18:0	18:1	18:2 [LA]	18:3 (ω3) [ALA]	20:2 [EDA]	20:3 (ω6) [DGLA]	20:3 (ω3) [ETrA]	20:4 (ω3) [ETA]	20:5 (ω3) [EPA]
pPEX10-1	27.1	3.9	25.0	25.2	8.2	6.4	0.9	5.2	3.5	10.7
pPEX10-2	28.5	4.3	25.4	24.5	7.6	6.4	1.0	5.3	3.4	10.8

[0278] The results in Table 9 showed that expression of Pex10 in Y4128U1 (Δpex10), either from the native *Y. lipolytica* Pex10 promoter or from the *Y. lipolytica* FBAINm promoter, reduced the percent of EPA back to the level of Y4086 while increasing the total lipid content (TFA % DCW) up to the level of Y4086 (see data of Table 6 for comparison). EPA content per gram of dry cell changed from 63.2 mg in the case of the control sample (i.e., cells carrying pFBAIn-MOD-1) to 31.5 mg in cells carrying pFBAIn-PEX10, 29 mg in cells carrying pPEX10-1 and 30.8 mg in cells carrying pPEX10-2. These results demonstrated that disruption of the ring-finger domain of Pex10 increased the amount of EPA but reduced the total lipid content in the cell.

[0279] Thus, the results in Table 9 showed that compared to Y4128U1 (Δpex10) transformant with control plasmid, all transformants with Pex10 expressing plasmids showed higher lipid content (TFAs % DCW) (>27% versus 22.8%), lower EPA % TFAs (ca. 10.8% versus 27.7%), and lower EPA % DCW (<3.1% versus 6.3%). Additionally, strain Y4128U1 (Δpex10) transformant with control plasmid, as compared to those transformants with Pex10 expressing plasmids, had a 2.5-fold increase in the amount of EPA relative to the total PUFAs (44% of the PUFAs [as a % TFAs] versus 17.5% (avg) of the PUFAs [as a % TFAs]) and a 1.5-fold increase in the amount of C20 PUFAs relative to the total PUFAs (67% of the PUFAs [as a % TFAs] versus 44% (avg) of the PUFAs [as a % TFAs]).

Example 6

Generation of Y4184U Strain to Produce EPA

[0280] *Y. lipolytica* strain Y4184U was used as the host in Example 7, infra. Strain Y4184U was derived from *Y. lipolytica* ATCC #20362, and is capable of producing EPA via expression of a Δ9 elongase/Δ8 desaturase pathway. The strain has a Ura− phenotype and its construction is described in Example 7 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference.

[0281] In summary, however, the development of strain Y4184U required the construction of strain Y2224, strain Y4001, strain Y4001U, strain Y4036, strain Y4036U and strain Y4069 (supra, Example 1). Further development of strain Y4184U (diagrammed in FIG. **7**B) required generation of strain Y4084, strain Y4084U1, strain Y4127 (deposited with the American Type Culture Collection on Nov. 29, 2007, under accession number ATCC PTA-8802), strain Y4127U2, strain Y4158, strain Y4158U1 and strain Y4184. The plasmid construct pZKL1-2SP98C, used for transformation of strain Y4127U2, is diagrammed in FIG. **8**A (SEQ ID NO:54; described in Table 23 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference). Plasmid pZKL2-5U89GC, used for transformation of strain Y4158U1, is shown in FIG. **8**B (SEQ ID NO:55; described in Table 24 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference).

[0282] The final genotype of strain Y4184 (producing 31% EPA of total lipids) with respect to wildtype *Yarrowia lipolytica* ATCC #20362 was unknown 1-, unknown 2-, unknown 4-, unknown 5-, unknown 6-, unknown 7-, YAT1::ME3S::Pex16, EXP1::ME3S::Pex20 (2 copies), GPAT::EgD9e::Lip2, FBAINm::EgD9eS::Lip2, EXP1::EgD9eS::Lip1, FBA::EgD9eS::Pex20, YAT1::EgD9eS::Lip2, GPD::EgD9eS::Lip2, GPDIN::EgD8M::Lip1, YAT1::EgD8M::Aco, EXP1::EgD8M::Pex16, FBAINm::EgD8M::Pex20, FBAIN::EgD8M::Lip1 (2 copies), GPM/FBAIN::FmD12S::Oct, EXP1::FmD12S::Aco, YAT1::FmD12::Oct, GPD::FmD12::Pex20, EXP1::EgD5S::Pex20, YAT1::EgD5S::Aco, YAT1::Rd5S::Oct, FBAIN::EgD5::Aco, FBAINm::PaD17::Aco, EXP1::PaD17::Pex16, YAT1::PaD17S::Lip1, YAT1::YICPT1::Aco, GPD::YICPT1::Aco (wherein FmD12 is a *Fusarium moniliforme* Δ12 desaturase gene [Int'l. App. Pub. No. WO 2005/047485]; FmD12S is a codon-optimized Δ12 desaturase gene, derived from *Fusarium moniliforme* [Int'l. App. Pub. No. WO 2005/047485]; ME3S is a codon-optimized $C_{16/18}$ elongase gene, derived from *Mortierella alpina* [Int'l. App. Pub. No. WO 2007/046817]; EgD9e is a *Euglena gracilis* Δ9 elongase gene [Int'l. App. Pub. No. WO 2007/061742]; EgD9eS is a codon-optimized Δ9 elongase gene, derived from *Euglena gracilis* [Int'l. App. Pub. No. WO 2007/061742]; EgD8M is a synthetic mutant Δ8 desaturase [Int'l. App. Pub. No. WO 2008/073271], derived from *Euglena gracilis* [U.S. Pat. No. 7,256,033]; EgD5 is a *Euglena gracilis* Δ5 desaturase [U.S. Pat. App. Pub. US 2007-0292924-A1]; EgD5S is a codon-optimized Δ5 desaturase gene, derived from *Euglena gracilis* [U.S. Pat. App. Pub. No. 2007-0292924]; RD5S is a codon-optimized Δ5 desaturase, derived from *Peridinium* sp. CCMP626 [U.S. Pat. App. Pub. No. 2007-0271632]; PaD17 is a *Pythium* aphanidermatum Δ17 desaturase [Int'l. App. Pub. No. WO 2008/054565]; PaD17S is a codon-optimized Δ17 desaturase, derived from *Pythium* aphanidermatum [Int'l. App. Pub. No. WO 2008/054565]; and, YICPT1 is a *Yarrowia lipolytica* diacylglycerol cholinephosphotransferase gene [Int'l. App. Pub. No. WO 2006/052870]).

[0283] In order to disrupt the Ura3 gene in strain Y4184, construct pZKUE3S (FIG. **5**A; SEQ ID NO:31; described in Table 22 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) was used to integrate a EXP1::ME3S::Pex20 chimeric gene into the Ura3 gene of strain Y4184 to result in strains Y4184U1 (11.2% EPA of total lipids), Y4184U2 (10.6% EPA of total lipids) and Y4184U4 (15.5% EPA of total lipids), respectively (collectively, Y4184U).

Example 7

Chromosomal Deletion of Pex10 in *Yarrowia lipolytica Strain* Y4184U4 Increases Accumulation of EPA and Total Lipid Content

[0284] Construct pYPS161 (FIG. **9**A, SEQ ID NO:56) was used to knock out the chromosomal Pex10 gene from the EPA-producing *Yarrowia* strain Y4184U4 (Example 6). Transformation of *Y. lipolytica* strain Y4184U4 with the Pex10 knock out construct resulted in creation of strain Y4184 (□pex10). The effect of the Pex10 knockout on total oil and on EPA level was determined and compared. Specifically, knockout of Pex10 resulted in an increased percentage of EPA (as % TFAs and % DCW) and an increased total lipid content in the cell.

Construct PYSP161

[0285] The construct pYPS161 contained the following components:

TABLE 10

Description of Plasmid pYPS161 (SEQ ID NO: 56)

RE Sites And Nucleotides Within SEQ ID	Description Of Fragment And Chimeric Gene
NO: 56	Components
AscI/BsiWI (1521-157)	1364 bp Pex10 knockout fragment #1 of *Yarrowia* Pex10 gene (GenBank Accession No. AB036770)
PacI/SphI (5519-4229)	1290 bp Pex10 knockout fragment #2 of *Yarrowia* Pex10 gene (GenBank Accession No. AB036770)
SalI/EcoRI (7170-5551)	*Yarrowia* URA3 gene (GenBank Accession No. AJ306421)
2451-1571	ColE1 plasmid origin of replication
3369-2509	ampicillin-resistance gene (Amp^R) for selection in *E. coli*
3977-3577	*E. coli* f1 origin of replication

Generation of *Yarrowia lipolytica* Knockout Strain Y4184 (ΔPex10)

[0286] Standard protocols were used to transform *Yarrowia lipolytica* strain Y4184U4 (Example 6) with the purified 5.3 kB AscI/SphI fragment of Pex10 knockout construct pYPS161 (supra), and a cells alone control was also prepared. There were about 200 to 250 colonies present for each of the experimental transformations, while there were no colonies present on the cells alone plates (per expectations).

[0287] Colony PCR was used to screen for cells having the Pex10 deletion. Specifically, the PCR reaction was performed using MasterAmp Taq polymerase (Epicentre Technologies, Madison, Wis.) following standard protocols, using PCR primers Pex-10 del1 3'.Forward (SEQ ID NO:57) and Pex-10 del2 5'.Reverse (SEQ ID NO:58). The PCR reaction conditions were 94° C. for 5 min, followed by 30 cycles at 94° C. for 30 sec, 60° C. for 30 sec and 72° C. for 2 min, followed by a final extension at 72° C. for 6 min. The reaction was then held at 4° C. If the Pex10 knockout construct integrated within the Pex10 region, a single PCR product 2.8 kB in size was expected to be produced. In contrast, if the strain integrated the Pex10 knockout construct in a chromosomal region other than the Pex10 region, then two PCR fragments, i.e., 2.8 kB and 1.1 kB, would be generated. Of the 288 colonies screened, the majority had the Pex10 knockout construct integrated at a random site. Only one of the 288 colonies contained the Pex10 knockout. This strain was designated Y4184 (Δpex10).

Evaluation of *Yarrowia lipolytica* Strains Y4184 And Y4184 (ΔPex10) for Total Oil and EPA Production

[0288] To evaluate the effect of the Pex10 knockout on the percent of PUFAs in the total lipid fraction and the total lipid content in the cells, strains Y4184 and Y4184 (Δpex10) were grown under comparable oleaginous conditions. Specifically, cultures were grown at a starting OD_{600} of ~0.1 in 25 mL of either fermentation media (FM) or FM medium without Yeast Extract (FM without YE) in a 250 mL flask for 48 hrs. The cells were harvested by centrifugation for 10 min at 8000 rpm in a 50 mL conical tube. The supernatant was discarded and the cells were re-suspended in 25 mL of HGM and transferred to a new 250 mL flask. The cells were incubated with aeration for an additional 120 hrs at 30° C.

[0289] To determine the dry cell weight (DCW), the cells from 5 mL of the FM-grown cultures and 10 mL of the FM without YE-grown cultures were processed. The cultured cells were centrifuged for 10 min at 4300 rpm. The pellet was re-suspended using 10 mL of saline and was centrifuged under the same conditions for a second time. The pellet was then re-suspended using 1 mL of sterile H_2O (three times) and was transferred to a pre-weighed aluminum pan. The cells were dried overnight in a vacuum oven at 80° C. The weight of the cells was determined.

[0290] The total lipid content and fatty acid composition of transformants carrying the above plasmids were analyzed as described in Example 3. DCW, total lipid content (TFAs % DCW), total EPA % TFAs, and EPA % DCW are shown below in Table 11.

TABLE 11

Lipid Composition in *Y. lipolytica* Strains Y4184 And Y4184 (ΔPex10)

Media	Strain	DCW	TFAs % DCW	EPA % TFAs	EPA % DCW
FM	Y4184	11.5	11.8	20.6	2.4
	Y4184 (ΔPex10)	11.5	17.6	43.2	7.6
FM without YE	Y4184	4.6	8.8	23.2	2.0
	Y4184 (ΔPex10)	4.0	13.2	46.1	6.1

[0291] The results in Table 11 showed that knockout of the chromosomal Pex10 gene in Y4184 (ΔPex10) increased the percent of EPA (as % TFAs and as % DCW) and increased the total oil content, as compared to the percent of EPA and total oil content in strain Y4184 whose native Pex10p had not been knocked out. More specifically, in FM media, there was about 109% increase in EPA (% TFAs), about 216% increase in EPA productivity (% DCW) and about 49% increase in total oil (TFAs % DCW). In FM without YE media, there was about 100% increase in EPA (% TFAs), about 205% increase in EPA productivity (% DCW) and about 50% increase in total oil (TFAs % DCW).

[0292] Thus, the results in Table 11 showed that in FM medium, compared to the parent strain Y4184, Y4184 (ΔPex10) strain had higher lipid content (TFAs % DCW) (17.6% versus 11.8%), higher EPA % TFAs (43.2% versus 20.6%), and higher EPA % DCW (7.6% versus 2.4%). Similarly, in FM medium without YE, compared to the parent strain Y4184, Y4184 (ΔPex10) strain had higher lipid content (TFAs % DCW) (13.2% versus 8.8%), higher EPA % TFAs (46.1% versus 23.2%), and higher EPA % DCW (6.1% versus 2.0%).

Example 8

Prophetic

Chromosomal Knockout of Alternate Pex Genes in PUFA-Producing Strains Of *Yarrowia lipolytica*

[0293] The present Example describes various strains of *Yarrowia lipolytica* that have been engineered to produce ω-3/ω-6 PUFAs. It is contemplated that any of these *Y. lipolytica* host strains could be engineered to produce an increased amount of ω-3/ω-6 PUFAs in the total lipid fraction and in the oil fraction, if the chromosomal gene encoding Pex1p, Pex2p, Pex3p, Pex3Bp, Pex4p, Pex5p, Pex6p, Pex7p, Pex8p, Pex12p, Pex13p, Pex14p, Pex16p, Pex17p, Pex19p, Pex20p, Pex22p or Pex26p was disrupted using the methodology of Example 7, supra.

[0294] More specifically, a variety of *Yarrowia lipolytica* strains have been engineered by the Applicant's Assignee to produce high concentrations of various ω-3/ω-6 PUFAs via expression of a heterologous Δ6 desaturase/Δ6 elongase PUFA pathway or a heterologous Δ9 elongase/Δ8 desaturase PUFA pathway.

Summary of Representative *Yarrowia lipolytica* Strains Producing ω-3/ω-6 PUFAs

[0295] Although some representative strains are summarized in the Table below, the disclosure of *Yarrowia lipolytica* strains producing ω-3/ω-6 PUFAs is not limited in any way to the strains therein. Instead, all of the teachings provided in the present Application, in addition to the following commonly owned and co-pending applications, are useful for development of a suitable *Yarrowia lipolytica* strain engineered to produce ω-3/ω-6 PUFAs. These specifically include the following Applicants' Assignee's co-pending patents and applications: U.S. Pat. No. 7,125,672, U.S. Pat. No. 7,189,559, U.S. Pat. No. 7,192,762, U.S. Pat. No. 7,198,937, U.S. Pat. No. 7,202,356, U.S. Pat. No. 7,214,491, U.S. Pat. No. 7,238,482, U.S. Pat. No. 7,256,033, U.S. Pat. No. 7,259,255, U.S. Pat. No. 7,264,949, U.S. Pat. No. 7,267,976, U.S. Pat. No. 7,273,746, U.S. patent application Ser. No. 10/985,254 and No. 10/985,691 (filed Nov. 10, 2004), U.S. patent application Ser. No. 11/183,664 (filed Jul. 18, 2005), U.S. patent application Ser. No. 11/185,301 (filed Jul. 20, 2005), U.S. patent application Ser. No. 11/190,750 (filed Jul. 27, 2005), U.S. patent application Ser. No. 11/198,975 (filed Aug. 8, 2005), U.S. patent application Ser. No. 11/253,882 (filed Oct. 19, 2005), U.S. patent application Ser. No. 11/264,784 and No. 11/264,737 (filed Nov. 1, 2005), U.S. patent application Ser. No. 11/265,761 (filed Nov. 2, 2005), U.S. patent application Ser. No. 11/601,563 and No. 11/601,564 (filed Nov. 16, 2006), U.S. patent application Ser. No. 11/635,258 (filed Dec. 7, 2006), U.S. patent application Ser. No. 11/613,420 (filed Dec. 20, 2006), U.S. patent application Ser. No. 11/787,772 (filed Apr. 18, 2007), U.S. patent application Ser. No. 11/737,772 (filed Apr. 20, 2007), U.S. patent application Ser. No. 11/740,298 (filed Apr. 26, 2007), U.S. patent application Ser. No. 12/111,237 (filed Apr. 29, 2008), U.S. patent application Ser. No. 11/748,629 and No. 11/748,637 (filed May 15, 2007), U.S. patent application Ser. No. 11/779,915 (filed Jul. 19, 2007), U.S. Pat. App. No. 60/991,266 (filed Nov. 30, 2007), U.S. patent application Ser. No. 11/952,243 (filed Dec. 7, 2007), U.S. Pat. App. No. 61/041,716 (filed Apr. 2, 2008), U.S. patent application Ser. No. 12/061,738 (filed Apr. 3, 2008), U.S. patent application Ser. No. 12/099,811 (filed Apr. 9, 2008), U.S. patent application Ser. No. 12/102,879 (filed Apr. 15, 2008), U.S. patent application Ser. No. 12/111,237 (filed Apr. 29, 2008), U.S. Pat. App. No. 61/055,511 (filed May 23, 2008) and U.S. Pat. App. No. 61/093,007 (filed Aug. 29, 2008).

TABLE 12

Lipid Profile Of Representative *Yarrowia lipolytica* Strains Engineered To Produce ω-3/ω-6 PUF As

Strain	Reference	ATCC Deposit No.	Fatty Acid Content (As A Percent [%] of Total Fatty Acids) 16:0	16:1	18:0	18:1	18:2	18:3 (ALA)	GLA
Wildtype	US 2006-0035351-	#76982	14	11	3.5	34.8	31	—	0
pDMW208	A1; WO2006/033723	—	11.9	8.6	1.5	24.4	17.8	—	25.9
pDMW208D62		—	16.2	1.5	0.1	17.8	22.2	—	34
M4	US 2006-0115881-A1; WO2006/052870	—	15	4	2	5	27	—	35
Y2034	US 2006-0094092-	—	13.1	8.1	1.7	7.4	14.8	—	25.2
Y2047	A1; WO2006/055322	PTA-7186	15.9	6.6	0.7	8.9	16.6	—	29.7
Y2214		—	7.9	15.3	0	13.7	37.5	—	0
EU	US 2006-0115881-	—	19	10.3	2.3	15.8	12	—	18.7
Y2072	A1; WO2006/052870	—	7.6	4.1	2.2	16.8	13.9	—	27.8
Y2102		—	9	3	3.5	5.6	18.6	—	29.6
Y2088		—	17	4.5	3	2.5	10	—	20
Y2089		—	7.9	3.4	2.5	9.9	14.3	—	37.5
Y2095		—	13	0	2.6	5.1	16	—	29.1
Y2090		—	6	1	6.1	7.7	12.6	—	26.4
Y2096		PTA-7184	8.1	1	6.3	8.5	11.5	—	25
Y2201		PTA-7185	11	16.1	0.7	18.4	27	—	—
Y3000	US 2006-0110806-A1; WO2006/052871	PTA-7187	5.9	1.2	5.5	7.7	11.7	—	30.1
Y4001	WO2008/073367	—	4.3	4.4	3.9	35.9	23	0	—
Y4036		—	7.7	3.6	1.1	14.2	32.6	0	—
Y4070		—	8	5.3	3.5	14.6	42.1	0	—
Y4158		—	3.2	1.2	2.7	14.5	30.4	5.3	—
Y4184		—	3.1	1.5	1.8	8.7	31.5	4.9	—

TABLE 12-continued

Lipid Profile Of Representative *Yarrowia lipolytica* Strains Engineered To Produce ω-3/ω-6 PUF As

Strain	Fatty Acid Content (As A Percent [%] of Total Fatty Acids)							Lipid %
	20:2	DGLA	ARA	ETA	EPA	DPA	DHA	dcw
Wildtype	—	—	—	—	—	—	—	—
pDMW208	—	—	—	—	—	—	—	—
pDMW208D62	—	—	—	—	—	—	—	—
M4	—	8	0	0	0	—	—	—
Y2034	—	8.3	11.2	—	—	—	—	—
Y2047	—	0	10.9	—	—	—	—	—
Y2214	—	7.9	14	—	—	—	—	—
EU	—	5.7	0.2	3	10.3	—	—	36
Y2072	—	3.7	1.7	22	15	—	—	—
Y2102	—	3.8	2.8	2.3	18.4	—	—	—
Y2088	—	3	2.8	1.7	20	—	—	—
Y2089	—	2.5	1.8	1.6	17.6	—	—	—
Y2095	—	3.1	1.9	2.7	19.3	—	—	—
Y2090	—	6.7	2.4	3.6	26.6	—	—	22.9
Y2096	—	5.8	2.1	2.5	28.1	—	—	20.8
Y2201	3.3	3.3	1	3.8	9	—	—	—
Y3000	—	2.6	1.2	1.2	4.7	18.3	5.6	—
Y4001	23.8	0	0	0	—	—	—	
Y4036	15.6	18.2	0	0	—	—	—	
Y4070	6.7	2.4	11.9	—	—	—	—	
Y4158	6.2	3.1	0.3	3.4	20.5	—	—	27.3
Y4184	5.6	2.9	0.6	2.4	28.9	—	—	23.9

Chromosomal Knockout of Pex Genes

[0296] Following selection of a preferred *Yarrowia lipolytica* strain producing the desired ω-3/ω-6 PUFA (or combination of PUFAs thereof), one of skill in the art could readily engineer a suitable knockout construct, similar to pYPS161 in Example 7, to result in knockout of a chromosomal Pex gene upon transformation into the parental *Y. lipolytica* strain. Preferred Pex genes would include: YIPex1p (GenBank Accession No. CAG82178; SEQ ID NO:1), YIPex2p (GenBank Accession No. CAG77647; SEQ ID NO:2), YIPex3p (GenBank Accession No. CAG78565; SEQ ID NO:3), YIPex3Bp (GenBank Accession No. CAG83356; SEQ ID NO:4), YIPex4p (GenBank Accession No. CAG79130; SEQ ID NO:5), YIPex5p (GenBank Accession No. CAG78803; SEQ ID NO:6), YIPex6p (GenBank Accession No. CAG82306; SEQ ID NO:7), YIPex7p (GenBank Accession No. CAG78389; SEQ ID NO:8), YIPex8p (GenBank Accession No. CAG80447; SEQ ID NO:9), YIPex12p (GenBank Accession No. CAG81532; SEQ ID NO:11), YIPex13p (GenBank Accession No. CAG81789; SEQ ID NO:12), YIPex14p (GenBank Accession No. CAG79323; SEQ ID NO:13), YIPex16p (GenBank Accession No. CAG79622; SEQ ID NO:14), YIPex17p (GenBank Accession No. CAG84025; SEQ ID NO:15), YIPex19p (GenBank Accession No. AAK84827; SEQ ID NO:16), YIPex20p (GenBank Accession No. CAG79226; SEQ ID NO:17), YIPex22p (GenBank Accession No. CAG77876; SEQ ID NO:18) and YIPex26p (GenBank Accession No. NC_006072, antisense translation of nucleotides 117230-118387; SEQ ID NO:19).

[0297] It would be expected that the chromosomal disruption of Pex would result in an increased amount of PUFAs in the total lipid fraction and in the oil fraction, as a percent of total fatty acids, as compared with a eukaryotic organism whose native peroxisome biogenesis factor protein has not been disrupted, wherein the amount of PUFAs can be: 1) the PUFA that is the desired end product of a functional PUFA biosynthetic pathway, as opposed to PUFA intermediates or by-products, 2) C_{20} and C_{22} PUFAs, and/or 3) total PUFAs. Preferred results not only achieve an increase in the amount of PUFAs as a percent of total fatty acids but also result in an increased amount of PUFAs as a percent of dry cell weight, as compared with a eukaryotic organism whose native peroxisome biogenesis factor protein has not been disrupted. Again, the amount of PUFAs can be: 1) the PUFA that is the desired end product of a functional PUFA biosynthetic pathway, as opposed to PUFA intermediates or by-products, 2) the C_{20} and C_{22} PUFAs, and/or 3) the total PUFAs. In some cases, the total lipid content also increases, relative to that of a eukaryotic organism whose native peroxisome biogenesis factor protein has not been disrupted.

Example 9

Chromosomal Deletion of Pex16 In *Yarrowia lipolytica* Strain Y4036U Increases Percent DGLA Accumulated

[0298] The present Example describes use of construct pYRH13 (FIG. **9**B; SEQ ID NO:59) to knock out the chromosomal Pex16 gene in the DGLA-producing *Yarrowia* strain Y4036U (Example 1). Transformation of *Y. lipolytica* strain Y4036U with the Pex16 knockout construct resulted in creation of strain Y4036U (Δpex16). The effect of the Pex16 knockout on DGLA level was determined and compared. Specifically, knockout of Pex16 resulted in an increased percentage of DGLA as a percent of total fatty acids in the cell.

Construct pYRH13

[0299] Plasmid pYRH13 was derived from plasmid pYPS161 (FIG. **9**A, SEQ ID NO:56; Example 7). Specifi-

cally, a 1982 bp 5' promoter region of the *Yarrowia lipolytica* Pex16 gene (GenBank Accession No. CAG79622) replaced the AscI/BsiWI fragment of pYPS161 and a 448 bp 3' terminator region of the *Yarrowia lipolytica* Pex16 gene (GenBank Accession No. CAG79622) replaced the PacI/SphI fragment of pYPS161 to produce pYRH13 (SEQ ID NO:59; FIG. **9**B).

Generation of *Yarrowia lipotytica* Knockout Strain Y4036 (ΔPex16)

[0300] Standard protocols were used to transform *Yarrowia lipolytica* strain Y4036U (Example 1) with the purified 6.0 kB AscI/SphI fragment of Pex16 knockout construct pYRH13.

[0301] To screen for cells having the Pex16 deletion, colony PCR was performed using Taq polymerase (Invitrogen; Carlsbad, Calif.) and the PCR primers PEX16Fii (SEQ ID NO:60) and PEX16Rii (SEQ ID NO:61). This set of primers was designed to amplify a 1.1 kB region of the intact Pex16 gene, and therefore the Pex16 deleted mutant (i.e., Δpex16) would not produce the band. A second set of primers was designed to produce a band only when the Pex16 gene was deleted. Specifically, one primer (i.e., 3UTR-URA3; SEQ ID NO:62) binds to a region in the vector sequences of the introduced 6.0 kB AscI/SphI disruption fragment, and the other primer (i.e., PEX16-conf; SEQ ID NO:63) binds to the Pex16 terminator sequences of chromosome outside of the homologous region of the disruption fragment.

[0302] More specifically, the colony PCR was performed using a reaction mixture that contained: 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 400 μM each of dGTP, dCTP, dATP, and dTTP, 2 μM of each primer, 20 μl water and 2 U Taq polymerase. Amplification was carried out as follows: initial denaturation at 94° C. for 120 sec, followed by 35 cycles of denaturation at 94° C. for 60 sec, annealing at 55° C. for 60 sec, and elongation at 72° C. for 120 sec. A final elongation cycle at 72° C. for 5 min was carried out, followed by reaction termination at 4° C.

[0303] Of 205 colonies screened, 195 had the Pex16 knockout fragment integrated at a random site in the chromosome and thus were not Δpex16 mutants (however, the cells could grow on ura− plates, due to the presence of pYRH13). Three of these random integrants, designated as Y4036U-17, Y4036U-19 and Y4036U-33, were used as controls in lipid production experiments (infra).

[0304] The remaining 10 colonies screened (i.e., of the total 205) contained the Pex16 knockout. These ten Δpex16 mutants within the Y4036U strain background were designated RHY25 through RHY34.

Confirmation of *Yarrowia lipotytica* Knockout Strain Y4036U (ΔPex16) by Quantitative Real Time PCR

[0305] Further confirmation of the Pex16 knockout in strains RHY25 through RHY34 was performed by quantitative real time PCR, with the *Yarrowia* translation elongation factor (tef-1) gene (GenBank Accession No. AF054510) used as the control.

[0306] First, real time PCR primers and TaqMan probes targeting the Pex16 gene and the tef-1 gene, respectively, were designed with Primer Express software v 2.0 (Applied-Biosystems, Foster City, Calif.). Specifically, real time PCR primers ef-324F (SEQ ID NO:64), ef-392R (SEQ ID NO:65), PEX16-741F (SEQ ID NO:66) and PEX16-802R (SEQ ID NO:67) were designed, as well as the TaqMan probes ef-345T (i.e., 5' 6-FAM™-TGCTGGTGGTGTTGGTGAGTT-TAMRA™, wherein the nucleotide sequence is set forth as SEQ ID NO:68) and PEX16-760T (i.e., 5'-6FAM™-CT-GTCCATTCTGCGACCCCTC-TAMRA™, wherein the nucleotide sequence is set forth as SEQ ID NO:69). The 5' end of the TaqMan fluorogenic probes have the 6FAM™ fluorescent reporter dye bound, while the 3' end comprises the TAMRA™ quencher. All primers and probes were obtained from Sigma-Genosys (Woodlands, Tex.).

[0307] Knockout candidate DNA was prepared by suspending 1 colony in 50 μl of water. Reactions for tef-1 and PEX16 were run separately, in triplicate for each sample: Real time PCR reactions included 20 pmoles each of forward and reverse primers (i.e., ef-324F, ef-392R, PEX16-741F and PEX16-802R 5', supra), 5 pmoles TaqMan probe (i.e., ef-345T and PEX16-760T), 10 μl TaqMan Universal PCR Master Mix—No AmpErase® Uracil-N-Glycosylase (UNG) (Catalog No. PN 4326614, AppliedBiosystems), 1 μl colony suspension and 8.5 μl RNase/DNase free water for a total volume of 20 μl per reaction. Reactions were run on the ABI PRISM® 7900 Sequence Detection System under the following conditions: initial denaturation at 95° C. for 10 min, followed by 40 cycles of denaturation at 95° C. for 15 sec and annealing at 60° C. for 1 min. Real time data was collected automatically during each cycle by monitoring 6-FAM™ fluorescence. Data analysis was performed using tef-1 gene threshold cycle (CT) values for data normalization as per the ABI PRISM® 7900 Sequence Detection System instruction manual.

[0308] Based on this analysis, it was concluded that all ten of the Y4036U (Δpex16) colonies (i.e., RHY25 through RHY34) were valid Pex16 knockouts, wherein the pYRH13 construct had integrated into the chromosomal YIPex16.

Evaluation of *Yarrowia lipotytica Strains Y*4036U and Y4036U (ΔPex16) for DGLA Production

[0309] To evaluate the effect of the Pex16 knockout on the percent of PUFAs in the total lipid fraction and the total lipid content in the cells, the Y4036U and Y4036U (Δpex16) strains were grown under comparable oleaginous conditions. More specifically, strains Y4036U-17, Y4036U-19 and Y4036U-33 having the Pex16 knockout fragment integrated at a random site in the chromosome were considered as Pex16 wild type (i.e., Y4036U) and strains RHY25 through RHY34 were the Pex16 mutant strains (i.e., Y4036U (Δpex16)). Cultures of each strain were grown at a starting OD_{600} of ~0.1 in 25 mL of MM containing 90 mg/L L-leucine in a 125 mL flask for 48 hrs. The cells were harvested by centrifugation for 5 min at 4300 rpm in a 50 mL conical tube. The supernatant was discarded and the cells were re-suspended in 25 mL of HGM and transferred to a new 125 mL flask. The cells were incubated with aeration for an additional 120 hrs at 30° C.

[0310] The fatty acid composition (i.e., LA (18:2), ALA, EDA and DGLA) for each of the strains is shown below in Table 13; fatty acid composition is expressed as the weight percent (wt. %) of total fatty acids. The average fatty acid composition of strains Y4036U and Y4036U (Δpex16) are highlighted in gray and indicated with "Ave". None of the strains tested provided sufficient cell mass in MM+L-leucine media, and thus total lipid content was not analyzed.

TABLE 13

Lipid Composition In *Y. lipolytica* Strains Y4036U And Y4036U (Δpex16)

Strain	Sample	18:2	ALA	EDA	DGLA
Y4036U	Y4036U-17	26.1	2.4	9.9	24.9
	Y4036U-19	29.4	1.6	9.9	18.1
	Y4036U-33	20.7	3.1	11.2	27.3
Y4036U	AVE	25.4	2.4	10.3	23.4
Y4036U (Δpex16)	RHY25-1	14.9	5.1	5.5	44.1
	RHY25-2	14.3	5.0	5.4	42.6
	RHY26-1	14.4	5.1	5.6	42.9
	RHY26-2	13.8	4.9	5.9	44.6
	RHY27-1	14.4	5.0	5.4	42.6
	RHY27-2	15.1	4.9	5.6	44.2
	RHY28	15.3	4.6	5.7	42.6
	RHY29	15.4	4.8	5.5	43.9
	RHY30	15.5	4.5	5.9	43.6
	RHY31	15.5	4.7	5.8	43.9
	RHY32	15.5	4.9	5.9	44.4
	RHY33	15.9	4.7	5.9	41.8
	RHY34	15.9	4.9	6.2	43.5
Y4036U (Δpex16)	AVE	15.1	4.9	5.7	43.4

The results in Table 13 showed that knockout of the chromosomal Pex16 gene in Y4036U (Δpex16) increased the DGLA % TFAs approximately 85%, as compared to the DGLA % TFAs in strain Y4036U whose native Pex16p had not been knocked out. However, Y4036U (Δpex16) also had a ~40% decrease in the LA (18:2) accumulation.

[0311] Thus, the results in Table 13 showed that compared to the parent strain Y4036, Y4036 (ΔPex16) strain had higher average DGLA % TFAs (43.4% versus 23.4%). Additionally, strain Y4036U (Δpex16) had a 1.65-fold increase in the amount of DGLA relative to the total PUFAs (62.8% of the PUFAs [as a % TFAs] versus 38.1% of the PUFAs [as a % TFAs]) and a 1.3-fold increase in the amount of C20 PUFAs relative to the total PUFAs (71% of the PUFAs [as a % TFAs] versus 54.8% of the PUFAs [as a % TFAs]).

Example 10

Generation of Y4305 Strain to Produce about 53.2% EPA of Total Liquids

[0312] *Y. lipolytica* strain Y4305U, having a Ura− phenotype, was used as the host in Example 11, infra. Strain Y4305 (a Ura+ strain that was parent to Y4305U) was derived from *Y. lipolytica* ATCC #20362, and is capable of producing about 53.2% EPA relative to the total lipids via expression of a Δ9 elongase/Δ8 desaturase pathway.

[0313] The development of strain Y4305U required the construction of strain Y2224, strain Y4001, strain Y4001U, strain Y4036, strain Y4036U, strain Y4070 and strain Y4086 (supra, Example 1). Further development of strain Y4305U required construction of strain Y4086U1, strain Y4128 and strain Y4128U3 (supra, Example 2). Subsequently, development of strain Y4305U (diagrammed in FIG. **10**) required construction of strain Y4217 (producing 42% EPA), strain Y4217U2 (Ura−), strain Y4259 (producing 46.5% EPA), strain Y4259U2 (Ura−) and strain Y4305 (producing 53.2% EPA).

[0314] Although the details concerning transformation and selection of the EPA-producing strains developed after strain Y4128U3 are not elaborated herein, the methodology used for isolation of strain Y4217, strain Y4217U2, strain Y4259, strain Y4259U2, strain Y4305 and strain Y4305U was as described in Examples 1 and 2.

[0315] Briefly, construct pZKL2-5U89GC (FIG. **8**B; SEQ ID NO:55; described in Table 24 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) was generated to integrate one Δ9 elongase gene (i.e., EgD9eS), one Δ8 desaturase gene (i.e., EgD8M), one Δ5 desaturase gene (i.e., EgD5S), and one *Yarrowia lipolytica* diacylglycerol cholinephosphotransferase (CPT1) gene into the Lip2 loci (GenBank Accession No. AJ012632) of strain Y4128U3 to thereby enable higher level production of EPA. Six strains, designated as Y4215, Y4216, Y4217, Y4218, Y4219 and Y4220, produced about 41.1%, 41.8%, 41.7%, 41.1%, 41% and 41.1% EPA of total lipids, respectively.

[0316] Strain Y4217U1 and Y4217U2 were created by disrupting the Ura3 gene in strain Y4217 via construct pZKUE3S (FIG. **5**A; SEQ ID NO:31; described in Table 22 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference), comprising a chimeric EXP1::ME3S::Pex20 gene targeted for the Ura3 gene. Construct pZKL1-2SP98C (FIG. **8**A; SEQ ID NO:54; described in Table 23 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) was utilized to integrate one Δ9 elongase gene (i.e., EgD9eS), one Δ8 desaturase gene (i.e., EgD8M), one Δ12 desaturase gene (i.e., FmD12S), and one *Yarrowia lipolytica* CPT1 gene into the Lip1 loci (GenBank Accession No. Z50020) of strain Y4217U2, thereby resulting in isolation of strains Y4259, Y4260, Y4261, Y4262, Y4263 and Y4264, producing about 46.5%, 44.5%, 44.5%, 44.8%, 44.5% and 44.3% EPA of total lipids, respectively.

[0317] A Ura− derivative (i.e., strain Y4259U2) was then created, via transformation with construct pZKUM (FIG. **11**A; SEQ ID NO:70; described in Table 33 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference), which integrated a Ura3 mutant gene into the Ura3 gene of strain Y4259, thereby resulting in isolation of strains Y4259U1, Y4259U2 and Y4259U3, respectively (collectively, Y4259U) (producing 31.4%, 31% and 31.3% EPA of total lipids, respectively).

[0318] Finally, construct pZKD2-5U89A2 (FIG. **11**B; SEQ ID NO:71) was generated to integrate one Δ9 elongase gene, one Δ5 desaturase gene, one Δ8 desaturase gene, and one Δ12 desaturase gene into the diacylglycerol acyltransferase (DGAT2) loci of strain Y4259U2, to thereby enable increased production of EPA. The pZKD2-5U89A2 plasmid contained the following components:

TABLE 14

Description of Plasmid pZKD2-5U89A2 (SEQ ID NO: 71)

RE Sites And Nucleotides Within SEQ ID NO: 71	Description Of Fragment And Chimeric Gene Components
AscI/BsiWI (1-736)	728 bp 5' portion of *Yarrowia* DGAT2 gene (SEQ ID NO: 72) (labeled as "YLDGAT5" in Figure; U.S. Pat. No. 7,267,976)
PacI/SphI (4164-3444)	714 bp 3' portion of *Yarrowia* DGAT2 gene (SEQ ID NO: 72) (labeled as "YLDGAT3" in Figure; U.S. Pat. No. 7,267,976)
SwaI/BsiWI (13377-1)	YAT1::FmD12S::Lip2, comprising: YAT1: *Yarrowia lipolytica* YAT1 promoter (labeled as "YAT" in Figure; Pat. Appl. Pub. No. US 2006/0094102-A1);

TABLE 14-continued

Description of Plasmid pZKD2-5U89A2 (SEQ ID NO: 71)

RE Sites And Nucleotides Within SEQ ID NO: 71	Description Of Fragment And Chimeric Gene Components
	FmD12S: codon-optimized Δ12 elongase (SEQ ID NO: 74), derived from *Fusarium moniliforme* (labeled as "F.D12S" in Figure; Int'l. App. Pub. No. WO 2005/047485); Lip2: Lip2 terminator sequence from *Yarrowia* Lip2 gene (GenBank Accession No. AJ012632)
PmeI/SwaI (10740-13377)	FBAIN::EgD8M::Lip1 comprising: FBAIN: *Yarrowia lipolytica* FBAIN promoter (U.S. Pat. No. 7,202,356); EgD8M: Synthetic mutant Δ8 desaturase (SEQ ID NO: 76; Pat. Appl. Pub. No. US 2008-0138868 A1), derived from *Euglena gracilis* ("EgD8S"; U.S. Pat. No. 7,256,033); Lip1: Lip1 terminator sequence from *Yarrowia* Lip1 gene (GenBank Accession No. Z50020)
ClaI/PmeI (8846-10740)	YAT1::E389D9eS::OCT, comprising: YAT1: *Yarrowia lipolytica* YAT1 promoter (labeled as "YAT" in Figure; Pat. Appl. Pub. No. US 2006/0094102-A1); E389D9eS: codon-optimized Δ9 elongase (SEQ ID NO: 78), derived from *Eutreptiella* sp. CCMP389 (labeled as "D9ES-389" in Figure; Int'l. App. Pub. No. WO 2007/061742); OCT: OCT terminator sequence from *Yarrowia* OCT gene (GenBank Accession No. X69988)
ClaI/EcoRI (8846-6777)	*Yarrowia* Ura3 gene (GenBank Accession No. AJ306421)
EcoRI/PacI (6777-4164)	EXP1::EgD5S::ACO, comprising: EXP1: *Yarrowia lipolytica* export protein (EXP1) promoter (labeled as "Exp" in Figure; Int'l. App. Pub. No. WO 2006/052870); EgD5S: codon-optimized Δ5 desaturase (SEQ ID NO: 80), derived from *Euglena gracilis* (Pat. Appl. Pub. No. US 2007-0292924-A1); Aco: Aco terminator sequence from *Yarrowia* Aco gene (GenBank Accession No. AJ001300)

[0319] The pZKD2-5U89A2 plasmid was digested with AscI/SphI and then used for transformation of strain Y4259U2 according to the General Methods. The transformed cells were plated onto MM plates, and plates were maintained at 30° C. for 3 to 4 days. Single colonies were re-streaked onto MM plates, and the resulting colonies were used to inoculate liquid MM. Liquid cultures were shaken at 250 rpm/min for 2 days at 30° C. The cells were collected by centrifugation, resuspended in HGM, and then shaken at 250 rpm/min for 5 days. The cells were collected by centrifugation, and lipids were extracted. FAMEs were prepared by trans-esterification and subsequently analyzed with a Hewlett-Packard 6890 GC.

[0320] GC analyses showed that most of the selected 96 strains produced 40-46% EPA of total lipids. Four strains, designated as Y4305, Y4306, Y4307 and Y4308, produced about 53.2%, 46.4%, 46.8% and 47.8% EPA of total lipids, respectively. The complete lipid profile of Y4305 is as follows: 16:0 (2.8%), 16:1 (0.7%), 18:0 (1.3%), 18:1 (4.9%), 18:2 (17.6%), ALA (2.3%), EDA (3.4%), DGLA (2.0%), ARA (0.6%), ETA (1.7%) and EPA (53.2%). The total lipid % dry cell weight was 27.5.

[0321] The final genotype of strain Y4305 with respect to wild type *Yarrowia lipolytica* ATCC #20362 was SCP2-(YALI0E01298g), YALI0C18711g-, Pex10-, YALI0F24167g-, unknown 1-, unknown 3-, unknown 8-, GPD::FmD12::Pex20, YAT1::FmD12::OCT, GPM/FBAIN::FmD12S::OCT, EXP1::FmD12S::Aco, YAT1::FmD12S::Lip2, YAT1::ME3S::Pex16, EXP1::ME3S::Pex20 (3 copies), GPAT::EgD9e::Lip2, EXP1::EgD9eS::Lip1, FBAINm::EgD9eS::Lip2, FBA::EgD9eS::Pex20, GPD::EgD9eS::Lip2, YAT1::EgD9eS::Lip2, YAT1::E389D9eS::OCT, FBAINm::EgD8M::Pex20, FBAIN::EgD8M::Lip1 (2 copies), EXP1::EgD8M::Pex16, GPDIN::EgD8M::Lip1, YAT1::EgD8M::Aco, FBAIN::EgD5::Aco, EXP1::EgD5S::Pex20, YAT1::EgD5S::Aco, EXP1::EgD5S::ACO, YAT1::RD5S::OCT, YAT1::PaD17S::Lip1, EXP1::PaD17::Pex16, FBAINm::PaD17::Aco, YAT1::YICPT1::ACO, GPD::YICPT1::ACO.

[0322] In order to disrupt the Ura3 gene in strain Y4305, construct pZKUM (FIG. **11**A; SEQ ID NO:70; described in Table 33 of Int'l. App. Pub. No. WO 2008/073367, hereby incorporated herein by reference) was used to integrate a Ura3 mutant gene into the Ura3 gene of strain Y4305. A total of 8 transformants grown on MM+5-FOA plates were picked and re-streaked onto MM plates and MM+5-FOA plates, separately. All 8 strains had a Ura-phenotype (i.e., cells could grow on MM+5-FOA plates, but not on MM plates). The cells were scraped from the MM+5-FOA plates, and lipids were extracted. FAMEs were prepared by trans-esterification and subsequently analyzed with a Hewlett-Packard 6890 GC.

[0323] GC analyses showed the presence of 37.6%, 37.3% and 36.5% EPA of total lipids in pZKUM transformants #1, #6 and #7 grown on MM+5-FOA plates. These three strains were designated as strains Y4305U1, Y4305U2 and Y4305U3, respectively (collectively, Y4305U). For clarity in Example 11, strain Y4305U is referred to as strain Y4305U (Δpex10).

Example 11

Chromosomal Deletion of Pex16 in *Yarrowia lipolytica* Strain Y4305U (Δpex10) Further Increased Percent EPA Accumulated

The Double Pex10-Pex16 Knockout

[0324] The present Example describes use of construct pYRH13 (FIG. **9**B; SEQ ID NO:59) to knock out the chromosomal Pex16 in *Yarrowia* strain Y4305U (Δpex10) (Example 10), to thereby result in a Pex10-Pex16 double mutant. The effect of the Pex10-Pex16 double knockout on total oil and EPA level was determined and compared. Specifically, the effect of the Pex10-Pex16 double mutation in strain Y4305U (Δpex10) (Δpex16) resulted in an increased amount of EPA in the cell (EPA % TFAs and EPA % DCW), as compared to the single mutant (i.e., strain Y4305U (Δpex10)).

Generation of *Yarrowia lipolytica* Knockout Strain Y4305U (ΔPex10) (Δpex16)

[0325] Standard protocols were used to transform *Yarrowia lipolytica* strain Y4305U (Δpex10) (Example 10) with the purified 6.0 kB AscI/SphI fragment of Pex16 knockout construct pYRH13 (Example 9; SEQ ID NO:59). Screening and identification of cells having the Pex16 deletion was conducted by colony PCR, as described in Example 9.

[0326] Of 93 colonies screened, 88 had the Pex16 knockout fragment integrated at a random site in the chromosome and thus were not Δpex16 mutants (however, the cells could grow on Ura− plates, due to the presence of pYRH13). Two of these random integrants, designated as Y4305U-22 and Y4305U-25, were used as controls in lipid production experiments (infra).

[0327] The remaining 5 colonies screened (i.e., of the total 93) contained the Pex16 knockout. These five Δpex16 mutants within the Y4305U strain background were designated RHY20, RHY21, RHY22, RHY23 and RHY24. Further confirmation of the YIPex16 knockout was performed by quantitative real time PCR, as described in Example 9.

Evaluation of *Yarrowia lipolytica* Strains Y4305U (ΔPex10) and Y4305U (ΔPex10) (Δpex16) for EPA Production

[0328] To evaluate the effect of mutation in multiple Pex genes on the percent of PUFAs in the total lipid fraction and the total lipid content in the cells, Y4305U (Δpex10) and Y4305U (Δpex10) (Δpex16) strains were grown under comparable oleaginous conditions. More specifically, strains Y4305U-22 and Y4305U-25 having the Pex16 knockout fragment integrated at a random site in the chromosome were considered as Pex16 wild type, Pex10 knockouts (i.e., Y4305U (Δpex10)). Strains RHY22, RHY23 and RHY24 were the double knockout mutant strains (i.e., Y4305U (Δpex10) (Δpex16)). Cultures of each strain were grown in duplicate under comparable oleaginous conditions.

[0329] Specifically, cultures were grown at a starting OD_{600} of ~0.1 in 25 mL of synthetic dextrose media (SD) in a 125 mL flask for 48 hrs. The cells were harvested by centrifugation for 5 min at 4300 rpm in a 50 mL conical tube. The supernatant was discarded and the cells were re-suspended in 25 mL of HGM and transferred to a new 125 mL flask. The cells were incubated with aeration for an additional 120 hrs at 30° C.

[0330] To determine the dry cell weight (DCW), the cells from 5 mL of the HGM-grown cultures were processed. The cultured cells were centrifuged for 5 min at 4300 rpm. The pellet was re-suspended using 10 mL of sterile water and was centrifuged under the same conditions for a second time. The pellet was then re-suspended using 1 mL of sterile H_2O (three times) and was 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.

[0331] To determine the total lipid content, 1 mL of HGM cultured cells were collected by centrifugation for 1 min at 13,000 rpm, total lipids were extracted, and FAMEs were prepared by trans-esterification, and subsequently analyzed with a Hewlett-Packard 6890 GC (General Methods).

[0332] The fatty acid composition (i.e., 16:0 (palmitate), 16:1 (palmitoleic acid), 18:0, 18:1 (oleic acid), 18:2 (LA), 18:3 (ALA), EDA, DGLA, ARA, ETrA, ETA and EPA) for each of the strains is shown below in Table 15 (expressed as the weight percent (wt. %) of total fatty acids (TFA)), as well as the DCW (g/L) and total lipid content (TFAs % DCW). The average fatty acid composition of strains Y4305U (Δpex10) and Y4305U (Δpex10) (Δpex16) are highlighted in gray and indicated with "Ave".

TABLE 15

Lipid Composition In *Y. lipolytica* Strains Y4305U (ΔPex10) And Y4305U (ΔPex10) (ΔPex16)

Strain	Sample	DCW (g/L)	TFAs % DCW	16:0	16:1	18:0	18:1	18:2	18:3	EDA	DGLA	ARA	ETrA	ETA	EPA
Y4305U (Δpex10)	Y4305U -22 #1	3.50	29	3.1	0.7	2.1	6.4	18.7	2.6	4.2	1.8	0.5	1.8	2.0	45.4
	Y4305U -22 #2	3.94	29	3.0	0.7	2.1	6.2	18.5	2.5	4.5	1.8	0.5	1.8	2.0	45.6
	Y4305U -25 #1	4.14	31	3.6	1.1	1.8	6.1	18.8	2.4	4.5	1.9	0.6	1.6	2.0	43.9
	Y4305U -25 #2	4.12	30	3.6	1.1	1.8	6.1	18.7	2.4	4.6	1.9	0.6	1.6	2.0	44.0
Y4305U (Δpex10)	AVE	3.93	30	3.3	0.9	2.0	6.2	18.7	2.5	4.5	1.9	0.6	1.7	2.0	44.7
Y4305U (Δpex10) (Δpex16)	RHY22 #1	4.04	29	2.7	0.7	1.5	5.4	18.5	2.7	3.4	1.9	0.5	1.4	2.0	48.5
	RHY22 #2	3.82	32	2.7	0.6	1.5	5.5	18.4	3.0	3.0	2.0	0.5	1.5	2.0	48.8
	RHY23 #1	4.66	30	2.7	0.7	1.5	5.4	18.6	2.7	3.5	2.0	0.6	1.4	2.0	48.2
	RHY23 #2	4.18	30	2.7	0.7	1.5	5.4	18.4	2.6	3.5	1.9	0.6	1.4	2.0	48.5
	RHY24 #1	4.34	30	2.8	0.8	1.5	5.5	18.6	2.6	3.6	1.9	0.6	1.4	2.0	47.9
	RHY24 #2	4.58	30	2.7	0.7	1.5	5.6	18.8	2.6	3.6	2.0	0.6	1.4	2.0	47.8
Y4305U (Δpex10) (Δpex16)	AVE	4.27	30	2.7	0.7	1.5	5.5	18.6	2.7	3.4	2.0	0.6	1.4	2.0	48.3

[0333] The results in Table 15 showed that knockout of the chromosomal Pex16 gene in Y4305U (Δpex10) (Δpex16) increased the EPA % TFAs approximately 8%, as compared to the EPA % TFAs in strain Y4305U (Δpex1.0) whose native Pex16p had not been knocked out. Additionally, the EPA % DCW was also increased in the double mutant as compared to in the single mutant strain, while the TFAs % DCW remained the same.

[0334] Thus, the results in Table 15 showed that compared to the control Y4305 (ΔPex10) strains, Y4305 (ΔPex10, ΔPex16) strains on average had higher EPA % TFAs (48.3% versus 44.7%) and higher EPA % DCW (14.57% versus 13.23%). Strain Y4305 (ΔPex10, ΔPex16) had only a 1.05-fold increase in the amount of EPA relative to the total PUFAs (61% of the PUFAs [as a % TFAs] versus 58.3% of the PUFAs [as a % TFAs]) relative to strain Y4305 (ΔPex10), while the increase in the amount of C20 PUFAs relative to the total PUFAs was effectively identical (73% of the PUFAs [as a % TFAs] versus 72% of the PUFAs [as a % TFAs]).

Example 12

Chromosomal Deletion of Pex3 in *Yarrowia lipolytica* Strain Y4036U Increases Percent DGLA Accumulated

[0335] The present Example describes use of construct pY157 (FIG. **12**B; SEQ ID NO:82) to knock out the chromosomal Pex3 gene (SEQ ID NO:3) in the Ura-, DGLA-producing *Yarrowia* strain Y4036U (Example 1). Transformation of *Y. lipolytica* strain Y4036U with the Pex3 knockout construct resulted in creation of strain Y4036 (Δpex3). The effect of the Pex3 knockout on DGLA level was determined and compared to the control strain Y4036 (a Ura^+ strain that was parent to strain Y4036U). Specifically, knockout of Pex3 increased DGLA as a percentage of total fatty acids and improved ca. 3-fold DGLA % DCW, compared to the control.

Construct PY157

[0336] Plasmid pY87 (FIG. **12**A) contained a cassette to knock out the *Yarrowia lipolytica* diacylglycerol acyltransferase (DGAT2) gene, as described below in Table 16:

TABLE 16

Description of Plasmid pY87 (SEQ ID NO: 83)

RE Sites And Nucleotides Within SEQ ID NO: 83	Description Of Fragment And Chimeric Gene Components
SphI/PacI (1-721)	5' portion of *Yarrowia* DGAT2 gene (bases 1-720 of SEQ ID NO: 72) (U.S. Pat. No. 7,267,976)
PacI/BglII (721-2459)	LoxP::Ura3::LoxP, comprising: LoxP sequence (SEQ ID NO: 84); *Yarrowia* Ura3 gene (GenBank Accession No. AJ306421); LoxP sequence (SEQ ID NO: 84)
BglII/AscI (2459-3203)	3' portion of *Yarrowia* DGAT2 gene (bases 2468-3202 of SEQ ID NO: 72) (U.S. Pat. No. 7,267,976)
AscI/SphI (3203-5910)	Vector backbone including: ColE1 plasmid origin of replication; ampicillin-resistance gene (Amp^R) for selection in *E. coli* (4191-5051); *E. coli* f1 origin of replication

[0337] Plasmid pY157 was derived from plasmid pY87. Specifically, a 704 bp 5' promoter region of the *Yarrowia lipolytica* Pex3 gene replaced the SphI/PacI fragment of pY87 and a 448 bp 3' terminator region of the *Yarrowia lipolytica* Pex3 gene replaced the Bg/II/IAscI fragment of pY87 to produce pY157 (SEQ ID NO:82; FIG. **12**B).

Generation of *Yarrowia lipolytica* Knockout Strain Y4036 (ΔPex3)

[0338] Standard protocols were used to transform *Yarrowia lipolytica* strain Y4036U (Example 1) with the purified 3648 bp AscI/SphI fragment of Pex3 knockout construct pY157 (supra).

[0339] To screen for cells having the Pex3 deletion, colony PCR was performed using Taq polymerase (Invitrogen; Carlsbad, Calif.) and the PCR primers UP 768 (SEQ ID NO:85) and LP 769 (SEQ ID NO:86). This set of primers was designed to amplify a 2039 bp wild type band of the intact Pex3 gene and 3719 bp knockout-specific band when the Pex3 gene was disrupted by targeted knockout.

[0340] More specifically, the colony PCR was performed using a MasterAmp Taq kit (Epicentre Technologies, Madison, Wis.; Catalog No. 82250) and the manufacturer's instructions in a 25 μl reaction comprising: 2.5 μl of 10× MasterAmp Taq buffer, 2.0 μl of 25 mM $MgCl_2$, 7.5 μl of 16× MasterAmp Enhancer, 2.5 μl of 2.5 mM dNTPs (TaKaRa Bio Inc., Otsu Shiga, Japan), 1.0 μl of 10 μM Upper primer, 1.0 μl of 10 μM Lower primer, 0.25 μl of MasterAmp Taq DNA polymerase and 19.75 μl of water. Amplification was carried out as follows: initial denaturation at 95° C. for 5 min, followed by 40 cycles of denaturation at 95° C. for 30 sec, annealing at 56° C. for 60 sec, and elongation at 72° C. for 4 min. A final elongation cycle at 72° C. for 10 min was carried out, followed by reaction termination at 4° C.

[0341] Of 48 colonies screened, 46 had the 2039 bp band expected from the wild type (i.e., undisrupted) Pex3 gene thus were not Δpex3 mutants. The remaining 2 colonies showed only a faint band of 2039 bp, suggesting that they were Δpex3 mutants with some contaminating untransformed cells present in the background. This was confirmed by streaking the 2 putative knockout colonies on selection plates to isolate single colonies. Then, genomic DNA was isolated from 3 single colonies from each putative knockout strain and screened by the same primer pair. i.e., UP 768 and LP 769 (SEQ ID NOs:85 and 86). This method was considered more sensitive than colony PCR. All three single colonies from both primary transformants lacked the 2039 bp wild type band and instead possessed the 3719 bp knockout-specific band. The two Δpex3 mutants within the Y4036U strain background were designated L134 and L135.

Evaluation of *Yarrowia lipolytica* Strains Y4036 And Y4036 (ΔPex3) for DGLA Production

[0342] To evaluate the effect of the Pex3 knockout on the percent of PUFAs in the total lipid fraction and the total lipid content in the cells, the Y4036 and Y4036 (Δpex3) strains were grown under comparable oleaginous conditions. Strains Y4036, L134 (i.e., Y4036 (Δpex3)) and L135 (i.e., Y4036 (Δpex3)) were inoculated into 25 mL of CSM-Ura and grown at 30° C. overnight in a shaker. The preculture was aliquoted into fresh 25 mL CSM-Ura flasks at a final OD_{600} of 0.4. Cultures were grown at 30° C. in shaker. After 48 hrs, the cells (which barely grew) were spun down and resuspended in fresh 25 mL CSM-Ura and continued to grow for 72 hrs. Cells were spun down, re-suspended in 25 mL of HGM, and continued to grow as above for 72 hrs. Cells were harvested by centrifugation, washed once in distilled water and resuspended in 25 mL water to give a final volume of 20.5 mL. An aliquot (1.5 mL) was used for lipid content, following extraction of the total lipids, preparation of FAMEs by base transesterification, and analysis by a Hewlett-Packard 6890 GC (General Methods). The remaining aliquot was dried down to measure dry cell weight (DCW), as described in Example 11.

[0343] The fatty acid composition (i.e., 16:0 (palmitate), 16:1 (palmitoleic acid), 18:0, 18:1 (oleic acid), 18:2 (LA), EDA and DGLA) for each of the strains is shown below in Table 17 (expressed as the weight percent (wt. %) of total fatty acids (TFA)), as well as the total lipid content (TFA % DCW). The conversion efficiency ("CE") was measured according to the following formula: ([product]/[substrate+product])*100, where 'product' includes the immediate product and all products in the pathway derived from it. Thus, the Δ12 desaturase conversion efficiency (Δ12% CE) was calculated as: ([LA+EDA+DGLA]/[18:1+LA+EDA+DGLA])*100; the Δ9 elongase conversion efficiency (Δ9 elo % CE) was calculated as: ([EDA+DGLA]/[LA+EDA+DGLA])*100; and, the Δ8 desaturase conversion efficiency (Δ8% CE) was calculated as: ([DGLA]/[EDA+DGLA])*100. The average fatty acid composition of strains Y4036, L134 and L135 are highlighted in gray and indicated with "Ave", while "S.D." indicates the Standard Deviation. As expected, the Δpex3 strains did not grow on plates with oleate as a sole source of carbon.

TABLE 17

Lipid Content And Composition In *Y. lipolytica* Strains Y4036 And Y4036 (ΔPex3)

Strain	Sample	TFA % DCW	16:0	16:1	18:0	18:1	18:2	EDA	DGLA	Δ12 % CE	Δ9 elo % CE	Δ8 % CE
Y4036	Y4036-1	6.1	10	7	1	14	29	9	19	80	49	69
	Y4036-2	3.7	11	6	1	14	30	8	20	81	48	70
	Y4036-3	4.1	11	5	1	15	31	8	19	80	47	70
	Avg.	4.7	10	6	1	14	30	8	19	80	48	70
	S.D.	1.3	0.3	0.9	0.1	0.3	0.7	0.3	0.2	0.3	0.9	0.8
Y4036 (Δpex3)	L134-1	6.2	7	5	1	8	12	10	45	89	83	81
	L134-2	5.4	7	5	1	8	11	10	47	90	83	82
	L134-3	6.7	6	5	1	8	12	11	46	90	83	82
	Avg.	6.1	7	5	1	8	12	10	46	90	83	82
	S.D.	0.6	0.5	0.1	0.1	0.3	0.1	0.1	1.0	0.5	0.4	0.3
Y4036 (Δpex3)	L135-1	4.2	7	5	1	8	12	11	45	89	82	81
	L135-2	6.5	6	5	1	8	12	10	47	90	83	82
	L135-3	7.1	7	5	1	8	12	10	46	90	83	82
	Avg.	5.9	7	5	1	8	12	10	46	90	83	81
	S.D.	1.6	0.6	0.1	0.1	0.3	0.1	0.1	1.1	0.5	0.4	0.5

[0344] The results in Table 17 showed that knockout of the chromosomal Pex3 gene in Y4036 (Δpex3) increased the DGLA % TFAs approximately 142%, as compared to the DGLA % TFAs in strain Y4036 whose native Pex3p had not been knocked out. Specifically, the Pex3 knockout increased DGLA levels from ca. 19% in Y4036 to 46% in Y4036 (Δpex3) strains, L134 and L135. Additionally, the Δ9 elongase percent conversion efficiency increased from ca. 48% in Y4036 to 83% in Y4036 (Δpex3) strains, L134 and L135; and, TFA % DCW increased from 4.7% to 6% in the strains L134 and L135. The LA % TFAs decreased from 30% to 12%. Pex3 deletion indeed increases the flux of fatty acids and thus the substrate availability for Δ9 elongation.

[0345] Thus, the results in Table 17 showed that compared to the parent strain Y4036, Y4036 (ΔPex3) strain had on average higher lipid content (TFAs % DCW) (ca. 6.0% versus 4.7%), higher DGLA % TFAs (46% versus 19%), and higher DGLA % DCW (ca. 2.8% versus 0.9%). Additionally, strain Y4036 (ΔPex3) had a 2-fold increase in the amount of DGLA relative to the total PUFAs (67.7% of the PUFAs [as a % TFAs] versus 33.3% of the PUFAs [as a % TFAs]) and a 1.7-fold increase in the amount of C20 PUFAs relative to the total PUFAs (82% of the PUFAs [as a % TFAs] versus 47% of the PUFAs [as a % TFAs]).

[0346] It is hypothesized that the improved DGLA productivity would also result in improved EPA productivity in *Yarrowia lipolytica* strains engineered for EPA production (e.g., *Y. lipolytica* strain Y4305U, as described in Example 10, and derivatives therefrom).

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        35                  40                  45

Pro Val Thr Val Ala Asp Trp Glu Ile Val Glu Leu His Ala Ala Tyr
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Pro Asp Ser Glu Val Val Val Ala Pro Lys Gln Arg Lys Lys Glu Glu
        115                 120                 125

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

Gln Leu Pro Pro Glu Phe His Gln Leu Lys Asn Val Ser Ile Lys Val
            180                 185                 190

Ile Thr Pro Pro Asn Leu Ala Ser Pro Gln Gln Ala Ala Ser Ile Ala
        195                 200                 205

Val Glu Glu Lys Ser Glu Glu Ser Leu Ser Gln Asn Lys Pro Pro Ser
    210                 215                 220

Ser Glu Pro Lys Val Glu Val Pro Pro Asp Ile Ile Asn Pro Ala Ser
225                 230                 235                 240

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

Ala Lys Leu Ser Tyr Ala Leu Ala Asp Ala Leu Gly Ile Pro Ser Ser
            260                 265                 270

Val Gly His Val Ile Arg Phe Glu Ser Ala Ser Lys Pro Leu Ser Gln
        275                 280                 285

Lys Pro Gly Ala Leu Val Ile His Arg Phe Ile Thr Lys Thr Val Gly
    290                 295                 300

Ala Ala Glu Gln Lys Ser Leu Arg Leu Lys Gly Glu Lys Asn Ala Asp
305                 310                 315                 320

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

Lys Leu Gln Met Leu Glu Gly Pro Ile Thr Asn Phe Gln Arg Leu Pro
            340                 345                 350

Pro Ile Pro Glu Leu Leu Pro Leu Gly Gly Val Ile Gly Leu Gln Asn
        355                 360                 365

Ser Glu Gly Trp Ile Gln Gly Gly Tyr Leu Gly Glu Glu Pro Ile Pro
    370                 375                 380

Phe Val Ser Gly Ser Glu Ile Leu Arg Ser Glu Ser Ser Leu Ser Pro
385                 390                 395                 400

Ser Asn Ile Glu Ser Glu Asp Lys Arg Val Val Gly Leu Asp Asn Met
                405                 410                 415

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

Val Tyr Gly Ser Arg Gly Ser Gly Lys Ser Ala Val Leu Asn His Ile
        435                 440                 445

Lys Lys Glu Cys Lys Val Ser His Thr His Thr Val Ser Ile Ala Cys
    450                 455                 460

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            500                 505                 510

Ser Arg Thr Arg Gln Leu Thr Gln Leu Phe Leu Glu Leu Ala Leu Pro
        515                 520                 525

Ile Met Lys Ser Arg His Val Ser Val Val Ala Ser Ala Gln Ala Lys
    530                 535                 540

Glu Ser Leu His Met Asn Leu Val Thr Gly His Val Phe Glu Glu Leu
545                 550                 555                 560

Phe His Leu Lys Ser Pro Asp Lys Glu Ala Arg Leu Ala Ile Leu Ser
                565                 570                 575

Glu Ala Val Lys Leu Met Asp Gln Asn Val Ser Phe Ser Gln Asn Asp
            580                 585                 590

Val Leu Glu Ile Ala Ser Gln Val Asp Gly Tyr Leu Pro Gly Asp Leu
        595                 600                 605

Trp Thr Leu Ser Glu Arg Ala Gln His Glu Met Ala Leu Arg Gln Ile
    610                 615                 620

Glu Ile Gly Leu Glu Asn Pro Ser Ile Gln Leu Ala Asp Phe Met Lys
625                 630                 635                 640

Ala Leu Glu Asp Phe Val Pro Ser Ser Leu Arg Gly Val Lys Leu Gln
                645                 650                 655

Lys Ser Asn Val Lys Trp Asn Asp Ile Gly Gly Leu Lys Glu Thr Lys
            660                 665                 670

Ala Val Leu Leu Glu Thr Leu Glu Trp Pro Thr Lys Tyr Ala Pro Ile
        675                 680                 685

Phe Ala Ser Cys Pro Leu Arg Leu Arg Ser Gly Leu Leu Leu Tyr Gly
    690                 695                 700

Tyr Pro Gly Cys Gly Lys Thr Tyr Leu Ala Ser Ala Val Ala Ala Gln
705                 710                 715                 720

Cys Gly Leu Asn Phe Ile Ser Ile Lys Gly Pro Glu Ile Leu Asn Lys
                725                 730                 735

Tyr Ile Gly Ala Ser Glu Gln Ser Val Arg Glu Leu Phe Glu Arg Ala
            740                 745                 750

Gln Ala Ala Lys Pro Cys Ile Leu Phe Phe Asp Glu Phe Asp Ser Ile
        755                 760                 765

Ala Pro Lys Arg Gly His Asp Ser Thr Gly Val Thr Asp Arg Val Val
    770                 775                 780

Asn Gln Met Leu Thr Gln Met Asp Gly Ala Glu Gly Leu Asp Gly Val
785                 790                 795                 800

Tyr Val Leu Ala Ala Thr Ser Arg Pro Asp Leu Ile Asp Pro Ala Leu
                805                 810                 815

Leu Arg Pro Gly Arg Leu Asp Lys Met Leu Ile Cys Asp Leu Pro Ser
            820                 825                 830

Tyr Glu Asp Arg Leu Asp Ile Leu Arg Ala Ile Val Asp Gly Lys Met
        835                 840                 845

His Leu Asp Gly Glu Val Glu Leu Glu Tyr Val Ala Ser Arg Thr Asp
    850                 855                 860

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Gly Asp Ala Lys Lys Asp Pro Ala Ala Val Gln Asn Glu Val Met Asn
        915                 920                 925

Ala Arg Ala Ala Val Ala Glu Lys Ala Arg Val Thr Ala Lys Leu Glu
    930                 935                 940

Ala Leu Phe Lys Gly Met Ser Val Gly Val Asp Asn Asp Asp Asp Lys
945                 950                 955                 960

Pro Arg Lys Lys Ala Val Val Val Ile Lys Pro Gln His Met Asn Lys
                965                 970                 975

Ser Leu Asp Glu Thr Ser Pro Ser Ile Ser Lys Lys Glu Leu Leu Lys
            980                 985                 990

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

Val Gly Gly Gly Tyr Leu Trp Ser Lys Ile Glu Gly Tyr Leu Leu Ala
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Arg Ser Glu Asp Asp Val Ala Thr Asp Gly Thr Ser Val Arg Gly Ala
    130                 135                 140

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

Tyr Ser Ala Ala Thr Leu Gly Asn Phe Val Ala Phe Leu Tyr Thr Gly
                165                 170                 175

Arg Tyr Ala Thr Val Ile Met Arg Leu Leu Arg Ile Arg Leu Val Pro
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Ser Gln Arg Thr Ser Ser Arg Gln Val Ser Tyr Glu Phe Gln Asn Arg
        195                 200                 205

Gln Leu Val Trp Asn Ala Phe Thr Glu Phe Leu Ile Phe Ile Leu Pro
    210                 215                 220

Leu Leu Gln Leu Pro Lys Leu Lys Arg Arg Ile Glu Arg Lys Leu Gln
225                 230                 235                 240

Ser Leu Asn Val Thr Arg Val Gly Asn Val Glu Glu Ala Ser Glu Gly
                245                 250                 255

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

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

Val Thr Asn Pro Tyr Gln Ala Asp Cys Gly His Val Tyr Cys Tyr Val
    290                 295                 300

Cys Leu Val Thr Lys Leu Ala Gln Gly Asp Gly Asp Gly Trp Asn Cys
305                 310                 315                 320

Tyr Arg Cys Ala Lys Gln Val Gln Lys Met Lys Pro Trp Val Asp Val
                325                 330                 335

Asp Glu Ala Ala Val Val Gly Ala Ala Glu Met His Glu Lys Val Asp
            340                 345                 350

Val Ile Glu His Ala Glu Asp Asn Glu Gln Glu Glu Glu Glu Phe Asp
        355                 360                 365

Asp Asp Asp Glu Asp Ser Asn Phe Gln Leu Met Lys Asp
    370                 375                 380


<210> SEQ ID NO 3
<211> LENGTH: 431
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(431)
<223> OTHER INFORMATION: YlPex3p; GenBank Accession No. CAG78565

<400> SEQUENCE: 3

Met Asp Phe Phe Arg Arg His Gln Lys Lys Val Leu Ala Leu Val Gly
1               5                   10                  15

Val Ala Leu Ser Ser Tyr Leu Phe Ile Asp Tyr Val Lys Lys Lys Phe
            20                  25                  30

Phe Glu Ile Gln Gly Arg Leu Ser Ser Glu Arg Thr Ala Lys Gln Asn
        35                  40                  45

Leu Arg Arg Arg Phe Glu Gln Asn Gln Gln Asp Ala Asp Phe Thr Ile
    50                  55                  60

Met Ala Leu Leu Ser Ser Leu Thr Thr Pro Val Met Glu Arg Tyr Pro
65                  70                  75                  80

Val Asp Gln Ile Lys Ala Glu Leu Gln Ser Lys Arg Arg Pro Thr Asp
                85                  90                  95

Arg Val Leu Ala Leu Glu Ser Ser Thr Ser Ser Ser Ala Thr Ala Gln
            100                 105                 110

Thr Val Pro Thr Met Thr Ser Gly Ala Thr Glu Glu Gly Glu Lys Ser
        115                 120                 125

Lys Thr Gln Leu Trp Gln Asp Leu Lys Arg Thr Thr Ile Ser Arg Ala
    130                 135                 140

Phe Ser Leu Val Tyr Ala Asp Ala Leu Leu Ile Phe Phe Thr Arg Leu
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145                 150                 155                 160

Gln Leu Asn Ile Leu Gly Arg Arg Asn Tyr Val Asn Ser Val Val Ala
                165                 170                 175

Leu Ala Gln Gln Gly Arg Glu Gly Asn Ala Glu Gly Arg Val Ala Pro
            180                 185                 190

Ser Phe Gly Asp Leu Ala Asp Met Gly Tyr Phe Gly Asp Leu Ser Gly
        195                 200                 205

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

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

Ser Glu Arg Val Glu Glu Ala Val Arg Arg Val Trp Asp Pro Val Ser
                245                 250                 255

Pro Lys Ala Glu Leu Gly Phe Asp Glu Leu Ser Glu Leu Ile Gly Arg
            260                 265                 270

Thr Gln Met Leu Ile Asp Arg Pro Leu Asn Pro Ser Ser Pro Leu Asn
        275                 280                 285

Phe Leu Ser Gln Leu Leu Pro Pro Arg Glu Gln Glu Glu Tyr Val Leu
    290                 295                 300

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

Arg Arg Leu Leu Asp Glu Thr Ala Asp Phe Ile Glu Ser Pro Asn Ala
                325                 330                 335

Ala Glu Val Ile Glu Arg Leu Val His Ser Gly Leu Ser Val Phe Met
            340                 345                 350

Asp Lys Leu Ala Val Thr Phe Gly Ala Thr Pro Ala Asp Ser Gly Ser
        355                 360                 365

Pro Tyr Pro Val Val Leu Pro Thr Ala Lys Val Lys Leu Pro Ser Ile
    370                 375                 380

Leu Ala Asn Met Ala Arg Gln Ala Gly Gly Met Ala Gln Gly Ser Pro
385                 390                 395                 400

Gly Val Glu Asn Glu Tyr Ile Asp Val Met Asn Gln Val Gln Glu Leu
                405                 410                 415

Thr Ser Phe Ser Ala Val Val Tyr Ser Ser Phe Asp Trp Ala Leu
            420                 425                 430


<210> SEQ ID NO 4
<211> LENGTH: 395
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(395)
<223> OTHER INFORMATION: YlPex3Bp; GenBank Accession No. CAG83356

<400> SEQUENCE: 4

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

Gly Leu Ile Ala Val Ala Tyr Val Val Ile Ser Tyr Thr Thr Lys Arg
            20                  25                  30

Leu Ile Glu Lys Gln Glu Gln Lys Leu Glu Glu Glu Arg Ala Lys Glu
        35                  40                  45

Arg Leu Lys Gln Leu Phe Ala Gln Thr Gln Asn Glu Ala Ala Phe His
    50                  55                  60
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Thr Ala Ser Val Leu Pro Gln Leu Cys Glu Gln Ile Met Glu Phe Val
65                  70                  75                  80

Ala Val Glu Lys Ile Ala Glu Gln Leu Gln Asn Met Arg Ala Glu Lys
                85                  90                  95

Arg Lys Lys Gln Asn Met Asp Asp Asp Lys His Ser Val Leu Ser Leu
            100                 105                 110

Gly Thr Glu Thr Thr Ala Ser Met Ala Asp Gly Gln Lys Met Ser Lys
        115                 120                 125

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

Thr Leu Ile Tyr Cys Val Ser Leu Leu Asn Tyr Leu Ile Arg Leu Gln
145                 150                 155                 160

Thr Asn Ile Val Gly Arg Lys Arg Tyr Gln Asn Glu Ala Gly Pro Ala
                165                 170                 175

Gly Ala Thr Tyr Asp Met Ser Leu Glu Gln Cys Tyr Thr Trp Leu Leu
            180                 185                 190

Thr Arg Gly Trp Lys Ser Val Val Asp Asn Val Arg Arg Ser Val Gln
        195                 200                 205

Gln Val Phe Thr Gly Val Asn Pro Arg Gln Asn Leu Ser Leu Asp Glu
    210                 215                 220

Phe Ala Thr Leu Leu Lys Arg Val Gln Thr Leu Val Asn Ser Pro Pro
225                 230                 235                 240

Tyr Ser Thr Thr Pro Asn Thr Phe Leu Thr Ser Leu Leu Pro Pro Arg
                245                 250                 255

Glu Leu Glu Gln Leu Arg Leu Glu Lys Glu Lys Gln Ser Leu Ser Pro
            260                 265                 270

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

Gln His Ile Gln Ser Pro Gln Gly Met Ser Ser Phe Arg Ala Ile Ile
    290                 295                 300

Asp Gln Ser Phe Lys Val Phe Leu Glu Lys Val Asn Glu Ser Gln Tyr
305                 310                 315                 320

Val Asn Pro Pro Ser Thr Gly Gly Lys Arg Ile Ala Val Gly Ala Leu
                325                 330                 335

Gln Pro Pro Ile Ile Ser Gly Gly Pro Lys Lys Val Lys Leu Ala Ser
            340                 345                 350

Leu Leu Ser Val Ala Thr Arg Gln Ser Ser Val Ile Ser His Ala Gln
        355                 360                 365

Pro Asn Pro Tyr Val Asp Ala Ile Asn Ser Val Ala Glu Tyr Asn Gly
    370                 375                 380

Leu Cys Ala Val Ile Tyr Ser Ser Phe Glu Gln
385                 390                 395


<210> SEQ ID NO 5
<211> LENGTH: 153
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(153)
<223> OTHER INFORMATION: YlPex4p; GenBank Accession No. CAG79130

<400> SEQUENCE: 5
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Met Ala Ser Gln Lys Arg Leu Ile Lys Glu Leu Ala Ala Tyr Lys Lys
1               5                   10                  15

Asp Pro Asn Pro Cys Leu Ala Ser Leu Thr Ala Asp Gly Asp Ser Leu
            20                  25                  30

Tyr Lys Trp Thr Ala Val Met Arg Gly Thr Glu Gly Thr Ala Tyr Glu
        35                  40                  45

Asn Gly Leu Trp Gln Val Glu Ile Asn Ile Pro Glu Asn Tyr Pro Leu
    50                  55                  60

Gln Pro Pro Thr Met Phe Phe Arg Thr Lys Ile Cys His Pro Asn Ile
65                  70                  75                  80

His Phe Glu Thr Gly Glu Val Cys Ile Asp Val Leu Lys Thr Gln Trp
                85                  90                  95

Ser Pro Ala Trp Thr Ile Ser Ser Ala Cys Thr Ala Val Ser Ala Met
            100                 105                 110

Leu Ser Leu Pro Glu Pro Asp Ser Pro Leu Asn Ile Asp Ala Ala Asn
        115                 120                 125

Leu Val Arg Cys Gly Asp Glu Ser Ala Met Glu Gly Leu Val Arg Tyr
    130                 135                 140

Tyr Val Asn Lys Tyr Ala Ser Gly Asn
145                 150


<210> SEQ ID NO 6
<211> LENGTH: 598
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(598)
<223> OTHER INFORMATION: YlPex5p; GenBank Accession No. CAG78803

<400> SEQUENCE: 6

Met Ser Phe Met Arg Gly Gly Ser Glu Cys Ser Thr Gly Arg Asn Pro
1               5                   10                  15

Leu Ser Gln Phe Thr Lys His Thr Ala Glu Asp Arg Ser Leu Gln His
            20                  25                  30

Asp Arg Val Ala Gly Pro Ser Gly Gly Arg Val Gly Gly Met Arg Ser
        35                  40                  45

Asn Thr Gly Glu Met Ser Gln Gln Asp Arg Glu Met Met Ala Arg Phe
    50                  55                  60

Gly Ala Ala Gly Pro Glu Gln Ser Ser Phe Asn Tyr Glu Gln Met Arg
65                  70                  75                  80

His Glu Leu His Asn Met Gly Ala Gln Gly Gly Gln Ile Pro Gln Val
                85                  90                  95

Pro Ser Gln Gln Gly Ala Ala Asn Gly Gly Gln Trp Ala Arg Asp Phe
            100                 105                 110

Gly Gly Gln Gln Thr Ala Pro Gly Ala Ala Pro Gln Asp Ala Lys Asn
        115                 120                 125

Trp Asn Ala Glu Phe Gln Arg Gly Gly Ser Pro Ala Glu Ala Met Gln
    130                 135                 140

Gln Gln Gly Pro Gly Pro Met Gln Gly Gly Met Gly Met Gly Gly Met
145                 150                 155                 160

Pro Met Tyr Gly Met Ala Arg Pro Met Tyr Ser Gly Met Ser Ala Asn
                165                 170                 175

Met Ala Pro Gln Phe Gln Pro Gln Gln Ala Asn Ala Arg Val Val Glu
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            180                 185                 190

Leu Asp Glu Gln Asn Trp Glu Glu Gln Phe Lys Gln Met Asp Ser Ala
        195                 200                 205

Val Gly Lys Gly Lys Glu Val Glu Glu Gln Thr Ala Glu Thr Ala Thr
    210                 215                 220

Ala Thr Glu Thr Val Thr Glu Thr Glu Thr Thr Thr Glu Asp Lys Pro
225                 230                 235                 240

Met Asp Ile Lys Asn Met Asp Phe Glu Asn Ile Trp Lys Asn Leu Gln
                245                 250                 255

Val Asn Val Leu Asp Asn Met Asp Glu Trp Leu Glu Glu Thr Asn Ser
            260                 265                 270

Pro Ala Trp Glu Arg Asp Phe His Glu Tyr Thr His Asn Arg Pro Glu
        275                 280                 285

Phe Ala Asp Tyr Gln Phe Glu Glu Asn Asn Gln Phe Met Glu His Pro
    290                 295                 300

Asp Pro Phe Lys Ile Gly Val Glu Leu Met Glu Thr Gly Gly Arg Leu
305                 310                 315                 320

Ser Glu Ala Ala Leu Ala Phe Glu Ala Ala Val Gln Lys Asn Thr Glu
                325                 330                 335

His Ala Glu Ala Trp Gly Arg Leu Gly Ala Cys Gln Ala Gln Asn Glu
            340                 345                 350

Lys Glu Asp Pro Ala Ile Arg Ala Leu Glu Arg Cys Ile Lys Leu Glu
        355                 360                 365

Pro Gly Asn Leu Ser Ala Leu Met Asn Leu Ser Val Ser Tyr Thr Asn
    370                 375                 380

Glu Gly Tyr Glu Asn Ala Ala Tyr Ala Thr Leu Glu Arg Trp Leu Ala
385                 390                 395                 400

Thr Lys Tyr Pro Glu Val Val Asp Gln Ala Arg Asn Gln Glu Pro Arg
                405                 410                 415

Leu Gly Asn Glu Asp Lys Phe Gln Leu His Ser Arg Val Thr Glu Leu
            420                 425                 430

Phe Ile Arg Ala Ala Gln Leu Ser Pro Asp Gly Ala Asn Ile Asp Ala
        435                 440                 445

Asp Val Gln Val Gly Leu Gly Val Leu Phe Tyr Gly Asn Glu Glu Tyr
    450                 455                 460

Asp Lys Ala Ile Asp Cys Phe Asn Ala Ala Ile Ala Val Arg Pro Asp
465                 470                 475                 480

Asp Ala Leu Leu Trp Asn Arg Leu Gly Ala Thr Leu Ala Asn Ser His
                485                 490                 495

Arg Ser Glu Glu Ala Ile Asp Ala Tyr Tyr Lys Ala Leu Glu Leu Arg
            500                 505                 510

Pro Ser Phe Val Arg Ala Arg Tyr Asn Leu Gly Val Ser Cys Ile Asn
        515                 520                 525

Ile Gly Cys Tyr Lys Glu Ala Ala Gln Tyr Leu Leu Gly Ala Leu Ser
    530                 535                 540

Met His Lys Val Glu Gly Val Gln Asp Asp Val Leu Ala Asn Gln Ser
545                 550                 555                 560

Thr Asn Leu Tyr Asp Thr Leu Lys Arg Val Phe Leu Gly Met Asp Arg
                565                 570                 575

Arg Asp Leu Val Ala Lys Val Gly Asn Gly Met Asp Val Asn Gln Phe
            580                 585                 590
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Arg Asn Glu Phe Glu Phe
        595


<210> SEQ ID NO 7
<211> LENGTH: 1024
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1024)
<223> OTHER INFORMATION: YlPex6p; GenBank Accession No. CAG82306

<400> SEQUENCE: 7

Met Pro Ser Ile Ser His Lys Pro Ile Thr Ala Lys Leu Val Ala Ala
1               5                   10                  15

Pro Asp Ala Thr Lys Leu Glu Leu Ser Ser Tyr Leu Tyr Gln Gln Leu
            20                  25                  30

Phe Ser Asp Lys Pro Ala Glu Pro Tyr Val Ala Phe Glu Ala Pro Gly
        35                  40                  45

Ile Lys Trp Ala Leu Tyr Pro Ala Ser Glu Asp Arg Ser Leu Pro Gln
    50                  55                  60

Tyr Thr Cys Lys Ala Asp Ile Arg His Val Ala Gly Ser Leu Lys Lys
65                  70                  75                  80

Phe Met Pro Val Val Leu Lys Arg Val Asn Pro Val Thr Ile Glu His
                85                  90                  95

Ala Ile Val Thr Val Pro Ala Ser Gln Tyr Glu Thr Leu Asn Thr Pro
            100                 105                 110

Glu Gln Val Leu Lys Ala Leu Glu Pro Gln Leu Asp Lys Asp Arg Pro
        115                 120                 125

Val Ile Arg Gln Gly Asp Val Leu Leu Asn Gly Cys Arg Val Arg Leu
    130                 135                 140

Cys Glu Pro Val Asn Gln Gly Lys Val Val Lys Gly Thr Thr Lys Leu
145                 150                 155                 160

Thr Val Ala Lys Glu Gln Glu Thr Ile Gln Pro Ala Asp Glu Ala Ala
                165                 170                 175

Asp Val Ala Phe Asp Ile Ala Glu Phe Leu Asp Phe Asp Thr Ser Val
            180                 185                 190

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

Ala Ile Pro Thr Pro Leu Ser Asp Arg Phe Asp Asp Cys Glu Ser Arg
    210                 215                 220

Gly Phe Val Lys Ser Glu Thr Met Ser Lys Leu Gly Val Phe Ser Gly
225                 230                 235                 240

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

Leu Phe Ala Tyr Pro Glu Pro Asn Thr Val Lys Tyr Asp Val Val Tyr
            260                 265                 270

Val Ser Pro Ile Leu Tyr His Asn Ile Gly Asp Lys Glu Ile Glu Val
        275                 280                 285

Thr Pro Asn Gly Glu Thr His Lys Ser Val Gly Glu Ala Leu Asp Ser
    290                 295                 300

Val Leu Glu Ala Ala Glu Glu Val Lys Leu Ala Arg Val Leu Gly Pro
305                 310                 315                 320
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Thr Thr Thr Asp Arg Thr Phe Gln Thr Ala Tyr His Ala Gly Leu Gln
                325                 330                 335

Ala Tyr Phe Lys Pro Val Lys Arg Ala Val Arg Val Gly Asp Leu Ile
            340                 345                 350

Pro Ile Pro Phe Asp Ser Ile Leu Ala Arg Thr Ile Gly Glu Asp Pro
        355                 360                 365

Glu Met Ser His Ile Pro Leu Glu Ala Leu Ala Val Lys Pro Asp Ser
    370                 375                 380

Val Ala Trp Phe Gln Val Thr Ser Leu Asn Gly Ser Glu Asp Pro Ala
385                 390                 395                 400

Ser Lys Gln Tyr Leu Val Asp Ser Ser Gln Thr Lys Leu Ile Glu Gly
                405                 410                 415

Gly Thr Thr Ser Ser Ala Val Ile Pro Thr Ser Val Pro Trp Arg Glu
            420                 425                 430

Tyr Leu Gly Leu Asp Thr Leu Pro Lys Phe Gly Ser Glu Phe Ala Tyr
        435                 440                 445

Ala Asp Lys Ile Arg Asn Leu Val Gln Ile Ser Thr Ser Ala Leu Ser
    450                 455                 460

His Ala Lys Leu Asn Thr Ser Val Leu Leu His Ser Ala Lys Arg Gly
465                 470                 475                 480

Val Gly Lys Ser Thr Val Leu Arg Ser Val Ala Ala Gln Cys Gly Ile
                485                 490                 495

Ser Val Phe Glu Ile Ser Cys Phe Gly Leu Ile Gly Asp Asn Glu Ala
            500                 505                 510

Gln Thr Leu Gly Thr Leu Arg Ala Lys Leu Asp Arg Ala Tyr Gly Cys
        515                 520                 525

Ser Pro Cys Val Val Val Leu Gln His Leu Glu Ser Ile Ala Lys Lys
    530                 535                 540

Ser Asp Gln Asp Gly Lys Asp Glu Gly Ile Val Ser Lys Leu Val Asp
545                 550                 555                 560

Val Leu Ala Asp Tyr Ser Gly His Gly Val Leu Leu Ala Ala Thr Ser
                565                 570                 575

Asn Asp Pro Asp Lys Ile Ser Glu Ala Ile Arg Ser Arg Phe Gln Phe
            580                 585                 590

Glu Ile Glu Ile Gly Val Pro Ser Glu Pro Gln Arg Arg Gln Ile Phe
        595                 600                 605

Ser His Leu Thr Lys Ser Gly Pro Gly Gly Asp Ser Ile Arg Asn Ala
    610                 615                 620

Pro Ile Ser Leu Arg Ser Asp Val Ser Val Glu Asn Leu Ala Leu Gln
625                 630                 635                 640

Ser Ala Gly Leu Thr Pro Pro Asp Leu Thr Ala Ile Val Gln Thr Thr
                645                 650                 655

Arg Leu Arg Ala Ile Asp Arg Leu Asn Lys Leu Thr Lys Asp Ser Asp
            660                 665                 670

Thr Thr Leu Asp Asp Leu Leu Thr Leu Ser His Gly Thr Leu Gln Leu
        675                 680                 685

Thr Pro Ser Asp Phe Asp Asp Ala Ile Ala Asp Ala Arg Gln Lys Tyr
    690                 695                 700

Ser Asp Ser Ile Gly Ala Pro Arg Ile Pro Asn Val Gly Trp Asp Asp
705                 710                 715                 720

Val Gly Gly Met Glu Gly Val Lys Lys Asp Ile Leu Asp Thr Ile Glu
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               725                 730                 735

Thr Pro Leu Lys Tyr Pro His Trp Phe Ser Asp Gly Val Lys Lys Arg
            740                 745                 750

Ser Gly Ile Leu Phe Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Leu
        755                 760                 765

Ala Lys Ala Ile Ala Thr Thr Phe Ser Leu Asn Phe Phe Ser Val Lys
    770                 775                 780

Gly Pro Glu Leu Leu Asn Met Tyr Ile Gly Glu Ser Glu Ala Asn Val
785                 790                 795                 800

Arg Arg Val Phe Gln Lys Ala Arg Asp Ala Lys Pro Cys Val Val Phe
                805                 810                 815

Phe Asp Glu Leu Asp Ser Val Ala Pro Gln Arg Gly Asn Gln Gly Asp
            820                 825                 830

Ser Gly Gly Val Met Asp Arg Ile Val Ser Gln Leu Leu Ala Glu Leu
        835                 840                 845

Asp Gly Met Ser Thr Ala Gly Gly Glu Gly Val Phe Val Val Gly Ala
    850                 855                 860

Thr Asn Arg Pro Asp Leu Leu Asp Glu Ala Leu Leu Arg Pro Gly Arg
865                 870                 875                 880

Phe Asp Lys Met Leu Tyr Leu Gly Ile Ser Asp Thr His Glu Lys Gln
                885                 890                 895

Gln Thr Ile Met Glu Ala Leu Thr Arg Lys Phe Arg Leu Ala Ala Asp
            900                 905                 910

Val Ser Leu Glu Ala Ile Ser Lys Arg Cys Pro Phe Thr Phe Thr Gly
        915                 920                 925

Ala Asp Phe Tyr Ala Leu Cys Ser Asp Ala Met Leu Asn Ala Met Thr
    930                 935                 940

Arg Thr Ala Asn Glu Val Asp Ala Lys Ile Lys Leu Leu Asn Lys Asn
945                 950                 955                 960

Arg Glu Glu Ala Gly Glu Glu Pro Val Ser Ile Arg Trp Trp Phe Asp
                965                 970                 975

His Glu Ala Thr Lys Ser Asp Ile Glu Val Glu Val Ala Gln Gln Asp
            980                 985                 990

Phe Glu Lys Ala Lys Asp Glu Leu  Ser Pro Ser Val Ser  Ala Glu Glu
        995                 1000                1005

Leu Gln  His Tyr Leu Lys Leu  Arg Gln Gln Phe Glu  Gly Gly Lys
    1010                1015                1020

Lys


<210> SEQ ID NO 8
<211> LENGTH: 356
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(356)
<223> OTHER INFORMATION: YlPex7p; GenBank Accession No. CAG78389

<400> SEQUENCE: 8

Met Leu Gly Phe Lys Thr Gln Gly Phe Asn Gly Tyr Ala Ala Asn Tyr
1               5                   10                  15

Ser Pro Phe Phe Asn Asp Lys Ile Ala Val Gly Thr Ala Ala Asn Tyr
            20                  25                  30
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Gly Leu Val Gly Asn Gly Lys Leu Phe Ile Leu Gly Ile Ser Pro Glu
        35                  40                  45

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

Asp Val Ala Trp Ser Glu Gln His Glu Asn His Val Ala Thr Ala Cys
65                  70                  75                  80

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

Leu Val Ser Phe Lys Glu His Thr Arg Glu Val Phe Ser Val Asn Trp
            100                 105                 110

Asn Met Ala Asn Lys Ala Leu Phe Cys Thr Ser Ser Trp Asp Ser Thr
        115                 120                 125

Ile Lys Ile Trp Thr Pro Glu Arg Thr Asn Ser Ile Met Thr Leu Gly
    130                 135                 140

Gln Pro Ala Pro Ala Gln Gly Thr Asn Ala Ser Ala His Ile Gly Arg
145                 150                 155                 160

Gln Thr Ala Pro Asn Gln Ala Ala Ala Gln Glu Cys Ile Tyr Ser Ala
                165                 170                 175

Lys Phe Ser Pro His Thr Asp Ser Ile Ile Ala Ser Ala His Ser Thr
            180                 185                 190

Gly Met Val Lys Val Trp Asp Thr Arg Ala Pro Gln Pro Leu Gln Gln
        195                 200                 205

Gln Phe Ser Thr Gln Gln Thr Glu Ser Gly Gly Pro Pro Glu Val Leu
    210                 215                 220

Ser Leu Asp Trp Asn Lys Tyr Arg Pro Thr Val Ile Ala Thr Gly Gly
225                 230                 235                 240

Val Asp Arg Ser Val Gln Val Tyr Asp Ile Arg Met Thr Gln Pro Ala
                245                 250                 255

Ala Asn Gln Pro Val Gln Pro Leu Ser Leu Ile Leu Gly His Arg Leu
            260                 265                 270

Pro Val Arg Gly Val Ser Trp Ser Pro His His Ala Asp Leu Leu Leu
        275                 280                 285

Ser Cys Ser Tyr Asp Met Thr Ala Arg Val Trp Arg Asp Ala Ser Thr
    290                 295                 300

Gly Gly Asn Tyr Leu Ala Arg Gln Arg Gly Gly Thr Glu Val Lys Cys
305                 310                 315                 320

Met Asp Arg His Thr Glu Phe Val Ile Gly Gly Asp Trp Ser Leu Trp
                325                 330                 335

Gly Asp Pro Gly Trp Ile Thr Thr Val Gly Trp Asp Gln Met Val Tyr
            340                 345                 350

Val Trp His Ala
        355


<210> SEQ ID NO 9
<211> LENGTH: 671
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(671)
<223> OTHER INFORMATION: YlPex8p; GenBank Accession No. CAG80447

<400> SEQUENCE: 9

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

Asn Leu Asp Leu Leu Ile Asn Asn Leu Arg Gly Ser Ser Thr Pro Gly
            20                  25                  30

Ala Ala Glu Val Asp Thr Arg Asp Ile Leu Gln Arg Ile Val Phe Ile
        35                  40                  45

Leu Pro Thr Ile Lys Asn Pro Leu Asn Leu Asp Leu Val Ile Lys Glu
    50                  55                  60

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

Tyr Gln Gln Leu Thr Asp Ala Phe Arg Ala Thr Ile Lys Arg Lys Ala
                85                  90                  95

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

Phe Gln Val Ile Thr Arg Phe Ala Gly Pro Gly Trp Lys Lys Leu Pro
        115                 120                 125

Leu Leu Ala Gly Leu Ile Leu Ala Asp Tyr Asp Ile Ser Ala Asp Gly
    130                 135                 140

Pro Thr Leu Glu Arg Lys Pro Gly Phe Pro Ser Lys Leu Lys His Leu
145                 150                 155                 160

Leu Lys Arg Glu Phe Val Thr Thr Phe Asp Gln Cys Leu Ser Ile Asp
                165                 170                 175

Thr Arg Asn Arg Ser Asp Ala Thr Lys Trp Val Pro Val Leu Ala Cys
            180                 185                 190

Ile Ser Ile Ala Gln Val Tyr Ser Leu Leu Gly Asp Val Ala Ile Asn
        195                 200                 205

Tyr Arg Arg Phe Leu Gln Val Gly Leu Asp Leu Ile Phe Ser Asn Tyr
    210                 215                 220

Gly Leu Glu Met Gly Thr Ala Leu Ala Arg Leu His Ala Glu Ser Gly
225                 230                 235                 240

Gly Asp Ala Thr Thr Ala Gly Gly Leu Ile Gly Lys Lys Leu Lys Glu
                245                 250                 255

Pro Val Val Ala Leu Leu Asn Thr Phe Ala His Ile Ala Ser Ser Cys
            260                 265                 270

Ile Val His Val Asp Ile Asp Tyr Ile Asp Arg Ile Gln Asn Lys Ile
        275                 280                 285

Ile Leu Val Cys Glu Asn Gln Ala Glu Thr Trp Arg Ile Leu Thr Ile
    290                 295                 300

Glu Ser Pro Thr Val Met His His Gln Glu Ser Val Gln Tyr Leu Lys
305                 310                 315                 320

Trp Glu Leu Phe Thr Leu Cys Ile Ile Met Gln Gly Ile Ala Asn Met
                325                 330                 335

Leu Leu Thr Gln Lys Met Asn Gln Phe Met Tyr Leu Gln Leu Ala Tyr
            340                 345                 350

Lys Gln Leu Gln Ala Leu His Ser Ile Tyr Phe Ile Val Asp Gln Met
        355                 360                 365

Gly Ser Gln Phe Ala Ala Tyr Asp Tyr Val Phe Phe Ser Ala Ile Asp
    370                 375                 380

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

Pro Pro Asn Lys Glu Phe Val Ala Glu Arg Leu Ala Ala Asn Leu Ala
                405                 410                 415
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```
Gly Thr Ser Asn Val Gly Ser His Leu Pro Ile Asp Arg Ser Arg Val
            420                 425                 430

Leu Phe Ala Leu Asn Tyr Tyr Glu Gln Leu Val Thr Val Cys His Asp
        435                 440                 445

Ser Cys Val Glu Thr Ile Ile Tyr Pro Met Ala Arg Ser Phe Leu Tyr
    450                 455                 460

Pro Thr Ser Asp Ile Gln Gln Leu Lys Pro Leu Val Glu Ala Ala His
465                 470                 475                 480

Ser Val Ile Leu Ala Gly Leu Ala Val Pro Thr Asn Ala Val Val Asn
                485                 490                 495

Ala Lys Leu Ile Pro Glu Tyr Met Gly Gly Val Leu Pro Leu Phe Pro
            500                 505                 510

Gly Val Phe Ser Trp Asn Gln Phe Val Leu Ala Ile Gln Ser Ile Val
        515                 520                 525

Asn Thr Val Ser Pro Pro Ser Glu Val Phe Lys Thr Asn Gln Lys Leu
    530                 535                 540

Phe Arg Leu Val Leu Asp Ser Leu Met Lys Lys Cys Arg Asp Thr Pro
545                 550                 555                 560

Val Gly Ile Pro Val Pro His Ser Val Thr Val Ser Gln Glu Gln Glu
                565                 570                 575

Asp Ile Pro Pro Thr Gln Arg Ala Val Val Met Leu Ala Leu Ile Asn
            580                 585                 590

Ser Leu Pro Tyr Val Asp Ile Arg Ser Phe Glu Leu Trp Leu Gln Glu
        595                 600                 605

Thr Trp Asn Met Ile Glu Ala Thr Pro Met Leu Ala Glu Asn Ala Pro
    610                 615                 620

Asn Lys Glu Leu Ala His Ala Glu His Glu Phe Leu Val Leu Glu Met
625                 630                 635                 640

Trp Lys Met Ile Ser Gly Asn Ile Asp Gln Arg Leu Asn Asp Val Ala
                645                 650                 655

Ile Arg Trp Trp Tyr Lys Lys Asn Ala Arg Val His Gly Thr Leu
            660                 665                 670


<210> SEQ ID NO 10
<211> LENGTH: 377
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(377)
<223> OTHER INFORMATION: YlPex10p; GenBank Accession No. CAG81606

<400> SEQUENCE: 10

Met Trp Gly Ser Ser His Ala Phe Ala Gly Glu Ser Asp Leu Thr Leu
1               5                   10                  15

Gln Leu His Thr Arg Ser Asn Met Ser Asp Asn Thr Thr Ile Lys Lys
            20                  25                  30

Pro Ile Arg Pro Lys Pro Ile Arg Thr Glu Arg Leu Pro Tyr Ala Gly
        35                  40                  45

Ala Ala Glu Ile Ile Arg Ala Asn Gln Lys Asp His Tyr Phe Glu Ser
    50                  55                  60

Val Leu Glu Gln His Leu Val Thr Phe Leu Gln Lys Trp Lys Gly Val
65                  70                  75                  80
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Arg Phe Ile His Gln Tyr Lys Glu Glu Leu Glu Thr Ala Ser Lys Phe
                85                  90                  95

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

Glu Tyr Thr Asn Leu Met Tyr Thr Ile Arg Asp Arg Thr Ala Leu Pro
        115                 120                 125

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

Tyr Leu Phe Val Arg Tyr Met Gly Lys Leu Arg Ala Lys Leu Met Arg
145                 150                 155                 160

Glu Tyr Pro His Leu Val Glu Tyr Asp Glu Asp Glu Pro Val Pro Ser
                165                 170                 175

Pro Glu Thr Trp Lys Glu Arg Val Ile Lys Thr Phe Val Asn Lys Phe
            180                 185                 190

Asp Lys Phe Thr Ala Leu Glu Gly Phe Thr Ala Ile His Leu Ala Ile
        195                 200                 205

Phe Tyr Val Tyr Gly Ser Tyr Tyr Gln Leu Ser Lys Arg Ile Trp Gly
    210                 215                 220

Met Arg Tyr Val Phe Gly His Arg Leu Asp Lys Asn Glu Pro Arg Ile
225                 230                 235                 240

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

Phe Val Gln Thr Gly Arg Glu Tyr Leu Gly Ala Leu Leu Glu Lys Ser
            260                 265                 270

Val Glu Lys Glu Ala Gly Glu Lys Glu Asp Glu Lys Glu Ala Val Val
        275                 280                 285

Pro Lys Lys Lys Ser Ser Ile Pro Phe Ile Glu Asp Thr Glu Gly Glu
    290                 295                 300

Thr Glu Asp Lys Ile Asp Leu Glu Asp Pro Arg Gln Leu Lys Phe Ile
305                 310                 315                 320

Pro Glu Ala Ser Arg Ala Cys Thr Leu Cys Leu Ser Tyr Ile Ser Ala
                325                 330                 335

Pro Ala Cys Thr Pro Cys Gly His Phe Phe Cys Trp Asp Cys Ile Ser
            340                 345                 350

Glu Trp Val Arg Glu Lys Pro Glu Cys Pro Leu Cys Arg Gln Gly Val
        355                 360                 365

Arg Glu Gln Asn Leu Leu Pro Ile Arg
    370                 375


<210> SEQ ID NO 11
<211> LENGTH: 408
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(408)
<223> OTHER INFORMATION: YlPex12p; GenBank Accession No. CAG81532

<400> SEQUENCE: 11

Met Asp Tyr Phe Ser Ser Leu Asn Ala Ser Gln Leu Asp Pro Asp Val
1               5                   10                  15

Pro Thr Leu Phe Glu Leu Leu Ser Ala Lys Gln Leu Glu Gly Leu Ile
            20                  25                  30

Ala Pro Ser Val Arg Tyr Ile Leu Ala Phe Tyr Ala Gln Arg His Pro
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        35                  40                  45

Arg Tyr Leu Leu Arg Ile Val Asn Arg Tyr Asp Glu Leu Tyr Ala Leu
    50                  55                  60

Phe Met Gly Leu Val Glu Tyr Tyr Asn Leu Lys Thr Trp Asn Ala Ser
65                  70                  75                  80

Phe Thr Glu Lys Phe Tyr Gly Leu Lys Arg Thr Gln Ile Leu Thr Asn
                85                  90                  95

Pro Ala Leu Arg Thr Arg Gln Ala Val Pro Asp Leu Val Glu Ala Glu
            100                 105                 110

Lys Arg Leu Ser Lys Lys Lys Ile Trp Gly Ser Leu Phe Phe Leu Ile
        115                 120                 125

Val Val Pro Tyr Val Lys Glu Lys Leu Asp Ala Arg Tyr Glu Arg Leu
    130                 135                 140

Lys Gly Arg Tyr Leu Ala Arg Asp Ile Asn Glu Glu Arg Ile Glu Ile
145                 150                 155                 160

Lys Arg Thr Gly Thr Ala Gln Gln Ile Ala Val Phe Glu Phe Asp Tyr
                165                 170                 175

Trp Leu Leu Lys Leu Tyr Pro Ile Val Thr Met Gly Cys Thr Thr Ala
            180                 185                 190

Thr Leu Ala Phe His Met Leu Phe Leu Phe Ser Val Thr Arg Ala Tyr
        195                 200                 205

Ser Ile Asp Asp Phe Leu Leu Asn Ile Gln Phe Ser Arg Met Thr Arg
    210                 215                 220

Tyr Asp Tyr Gln Met Glu Thr Gln Arg Asp Ser Arg Asn Ala Ala Asn
225                 230                 235                 240

Val Ala His Thr Met Lys Ser Ile Ser Glu Tyr Pro Val Ala Glu Arg
                245                 250                 255

Val Met Leu Leu Leu Thr Thr Lys Ala Gly Ala Asn Ala Met Arg Ser
            260                 265                 270

Ala Ala Leu Ser Gly Leu Ser Tyr Val Leu Pro Thr Ser Ile Phe Ala
        275                 280                 285

Leu Lys Phe Leu Glu Trp Trp Tyr Ala Ser Asp Phe Ala Arg Gln Leu
    290                 295                 300

Asn Gln Lys Arg Arg Gly Asp Leu Glu Asp Asn Leu Pro Val Pro Asp
305                 310                 315                 320

Lys Val Lys Gly Ala Asp Lys Leu Ala Glu Ser Val Ala Lys Trp Lys
                325                 330                 335

Glu Asp Thr Ser Lys Cys Pro Leu Cys Ser Lys Glu Leu Val Asn Pro
            340                 345                 350

Thr Val Ile Glu Ser Gly Tyr Val Phe Cys Tyr Thr Cys Ile Tyr Arg
        355                 360                 365

His Leu Glu Asp Gly Asp Glu Glu Thr Gly Gly Arg Cys Pro Val Thr
    370                 375                 380

Gly Gln Lys Leu Leu Gly Cys Arg Trp Gln Asp Asp Val Trp Gln Val
385                 390                 395                 400

Thr Gly Leu Arg Arg Leu Met Val
                405
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<210> SEQ ID NO 12
<211> LENGTH: 412
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica

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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(412)
<223> OTHER INFORMATION: YlPex13p; GenBank Accession No. CAG81789

<400> SEQUENCE: 12

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

Ala Ala Thr Ala Thr Pro Ala Ala Thr Ala Thr Pro Ala Ser Thr Asp
20 25 30

Ala Val Ser Ser Ser Ala Gly Ser Ala Thr Gly Ala Pro Glu Leu Pro
35 40 45

Ser Arg Pro Ser Ala Met Gly Ser Thr Ser Asn Ala Leu Ser Ser Pro
50 55 60

Met Gly Ser Ser Met Asn Ser Gly Tyr Gly Gly Met Asn Ser Gly Tyr
65 70 75 80

Gly Gly Met Gly Ser Ser Tyr Gly Ser Gly Tyr Gly Ser Ser Tyr Gly
85 90 95

Met Gly Ser Ser Tyr Gly Ser Gly Tyr Gly Ser Gly Leu Gly Gly Tyr
100 105 110

Gly Ser Tyr Gly Gly Met Gly Gly Met Gly Gly Met Tyr Gly Ser Arg
115 120 125

Tyr Gly Gly Tyr Gly Ser Tyr Gly Gly Met Gly Gly Tyr Gly Gly Tyr
130 135 140

Gly Gly Met Gly Gly Gly Pro Met Gly Gln Asn Gly Leu Ala Gly Gly
145 150 155 160

Thr Gln Ala Thr Phe Gln Leu Ile Glu Ser Ile Val Gly Ala Val Gly
165 170 175

Gly Phe Ala Gln Met Leu Glu Ser Thr Tyr Met Ala Thr Gln Ser Ser
180 185 190

Phe Phe Ala Met Val Ser Val Ala Glu Gln Phe Gly Asn Leu Lys Asn
195 200 205

Thr Leu Gly Ser Leu Leu Gly Ile Tyr Ala Ile Met Arg Trp Ala Arg
210 215 220

Arg Leu Val Ala Lys Leu Ser Gly Gln Pro Val Thr Gly Ala Asn Gly
225 230 235 240

Ile Thr Pro Ala Gly Phe Ala Lys Phe Glu Ala Thr Gly Gly Ala Ala
245 250 255

Gly Pro Gly Arg Gly Pro Arg Pro Ser Tyr Lys Pro Leu Leu Phe Phe
260 265 270

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

Ala Leu Ala Ala Lys Gln Glu Gly Met Tyr Asp Glu His Gly Asn Leu
290 295 300

Leu Pro Gly Ala Gln Met Gly Met Gly Gly Pro Gly Met Glu Gly Gly
305 310 315 320

Ala Glu Ile Asp Pro Ser Lys Leu Glu Phe Cys Arg Ala Asn Phe Asp
325 330 335

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

Leu Val Ala Val Leu Ala Lys Thr Asp Pro Met Gly Asn Pro Ser Gln
355 360 365

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Trp Trp Arg Val Arg Thr Arg Asp Gly Arg Ser Gly Tyr Val Pro Ala
    370                 375                 380

Asn Tyr Leu Glu Val Ile Pro Arg Pro Ala Val Glu Ala Pro Lys Lys
385                 390                 395                 400

Val Glu Glu Ile Gly Ala Ser Ala Val Pro Val Asn
                405                 410


<210> SEQ ID NO 13
<211> LENGTH: 380
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(380)
<223> OTHER INFORMATION: YlPex14p; GenBank Accession No. CAG79323

<400> SEQUENCE: 13

Met Ile Pro Ser Cys Leu Ser Thr Gln His Met Ala Pro Arg Glu Asp
1               5                   10                  15

Leu Val Gln Ser Ala Val Ala Phe Leu Asn Asp Pro Gln Ala Ala Thr
            20                  25                  30

Ala Pro Leu Ala Lys Arg Ile Glu Phe Leu Glu Ser Lys Asp Met Thr
        35                  40                  45

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

Gln Ser His Pro Gly Ser Val Val Ser His Gly Gly Ala Ala Pro Thr
65                  70                  75                  80

Val Pro Ala Ser Tyr Ala Phe Gln Ser Ala Pro Pro Leu Pro Glu Arg
                85                  90                  95

Asp Trp Lys Asp Val Phe Ile Met Ala Thr Val Thr Val Gly Val Gly
            100                 105                 110

Phe Gly Leu Tyr Thr Val Ala Lys Arg Tyr Leu Met Pro Leu Ile Leu
        115                 120                 125

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

Glu Phe Ala Arg Val Gln Gly Leu Leu Asp Gln Val Gln Gln Asp Thr
145                 150                 155                 160

Glu Glu Val Lys Asn Ser Gln Val Glu Val Ala Lys Arg Val Thr Asp
                165                 170                 175

Ala Leu Lys Gly Val Glu Glu Thr Ile Asp Gln Leu Lys Ser Gln Thr
            180                 185                 190

Lys Lys Arg Asp Asp Glu Met Lys Leu Val Thr Ala Glu Val Glu Arg
        195                 200                 205

Ile Arg Asp Arg Leu Pro Lys Asn Ile Asp Lys Leu Lys Asp Ser Gln
    210                 215                 220

Glu Gln Gly Leu Ala Asp Ile Gln Ser Glu Leu Lys Ser Leu Lys Gln
225                 230                 235                 240

Leu Leu Ser Thr Arg Thr Ala Ala Ser Ser Gly Pro Lys Leu Pro Pro
                245                 250                 255

Ile Pro Pro Pro Ser Ser Tyr Leu Thr Arg Lys Ala Ser Pro Ala Val
            260                 265                 270

Pro Ala Ala Ala Pro Ala Pro Val Thr Pro Gly Ser Pro Val His Asn
        275                 280                 285

Val Ser Ser Ser Ser Thr Val Pro Ala Asp Arg Asp Asp Phe Ile Pro
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    290                 295                 300

Thr Pro Ala Gly Ala Val Pro Met Ile Pro Gln Pro Ala Ser Met Ser
305                 310                 315                 320

Ser Ser Ser Thr Ser Thr Val Pro Asn Ser Ala Ile Ser Ser Ala Pro
                325                 330                 335

Ser Pro Ile Gln Glu Pro Glu Pro Phe Val Pro Glu Pro Gly Asn Ser
            340                 345                 350

Ala Val Lys Lys Pro Ala Pro Lys Ala Ser Ile Pro Ala Trp Gln Leu
        355                 360                 365

Ala Ala Leu Glu Lys Glu Lys Glu Lys Glu Lys Glu
    370                 375                 380


<210> SEQ ID NO 14
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(391)
<223> OTHER INFORMATION: YlPex16p; GenBank Accession No. CAG79622

<400> SEQUENCE: 14

Met Thr Asp Lys Leu Val Lys Val Met Gln Lys Lys Lys Ser Ala Pro
1               5                   10                  15

Gln Thr Trp Leu Asp Ser Tyr Asp Lys Phe Leu Val Arg Asn Ala Ala
            20                  25                  30

Ser Ile Gly Ser Ile Glu Ser Thr Leu Arg Thr Val Ser Tyr Val Leu
        35                  40                  45

Pro Gly Arg Phe Asn Asp Val Glu Ile Ala Thr Glu Thr Leu Tyr Ala
    50                  55                  60

Val Leu Asn Val Leu Gly Leu Tyr His Asp Thr Ile Ile Ala Arg Ala
65                  70                  75                  80

Val Ala Ala Ser Pro Asn Ala Ala Ala Val Tyr Arg Pro Ser Pro His
                85                  90                  95

Asn Arg Tyr Thr Asp Trp Phe Ile Lys Asn Arg Lys Gly Tyr Lys Tyr
            100                 105                 110

Ala Ser Arg Ala Val Thr Phe Val Lys Phe Gly Glu Leu Val Ala Glu
        115                 120                 125

Met Val Ala Lys Lys Asn Gly Gly Glu Met Ala Arg Trp Lys Cys Ile
    130                 135                 140

Ile Gly Ile Glu Gly Ile Lys Ala Gly Leu Arg Ile Tyr Met Leu Gly
145                 150                 155                 160

Ser Thr Leu Tyr Gln Pro Leu Cys Thr Thr Pro Tyr Pro Asp Arg Glu
                165                 170                 175

Val Thr Gly Glu Leu Leu Glu Thr Ile Cys Arg Asp Glu Gly Glu Leu
            180                 185                 190

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

Gly Arg Thr Ile Pro Glu Ile Ala Pro Thr Asn Val Glu Gly Tyr Leu
    210                 215                 220

Leu Thr Lys Val Leu Arg Ser Glu Asp Val Asp Arg Pro Tyr Asn Leu
225                 230                 235                 240

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

Lys Thr Val Pro Ala Ser Thr Lys Ser Lys Phe Pro Phe Leu Asn Ser
        275                 280                 285

Pro Trp Ala Pro Trp Ile Ile Gly Leu Val Ile Glu Ala Leu Ser Arg
    290                 295                 300

Lys Met Met Gly Ser Trp Leu Leu Arg Gln Arg Gln Ser Gly Lys Thr
305                 310                 315                 320

Pro Thr Ala Leu Asp Gln Met Glu Val Lys Gly Arg Thr Asn Leu Leu
                325                 330                 335

Gly Trp Trp Leu Phe Arg Gly Glu Phe Tyr Gln Ala Tyr Thr Arg Pro
            340                 345                 350

Leu Leu Tyr Ser Ile Val Ala Arg Leu Glu Lys Ile Pro Gly Leu Gly
        355                 360                 365

Leu Phe Gly Ala Leu Ile Ser Asp Tyr Leu Tyr Leu Phe Asp Arg Tyr
    370                 375                 380

Tyr Phe Thr Ala Ser Thr Leu
385                 390


<210> SEQ ID NO 15
<211> LENGTH: 225
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(225)
<223> OTHER INFORMATION: YlPex17p; GenBank Accession No. CAG84025

<400> SEQUENCE: 15

Met Ser Ala Phe Pro Glu Pro Ser Ser Phe Glu Ile Glu Phe Ala Lys
1               5                   10                  15

Gln Met Asn Arg Pro Arg Thr Val Gln Phe Lys Gln Leu Val Ala Val
            20                  25                  30

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

Thr Ile Leu Asn Pro Leu Phe Glu Glu Leu Thr Phe Ala Arg Ser Glu
    50                  55                  60

Tyr Ala Ile His Ala Arg Arg Leu Met Glu Gln Leu Asn Ala Lys Leu
65                  70                  75                  80

Ser Ser Met Ala Ser Tyr Ile Pro Pro Val Arg Ala Leu Gln Gly Gln
                85                  90                  95

Arg Phe Val Asp Ala Gln Thr Gln Thr Glu Asp Glu Glu Gly Glu Asp
            100                 105                 110

Ile Pro Asn Pro Ser Leu Gly Lys Ser Ser His Val Ser Phe Gly Glu
        115                 120                 125

Ser Pro Met Gln Leu Lys Leu Ala Glu Lys Glu Lys Gln Gln Lys Leu
    130                 135                 140

Ile Asp Asp Ser Val Asp Asn Leu Glu Arg Leu Ala Asp Ser Leu Lys
145                 150                 155                 160

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

Gln Val Glu Glu Leu Thr Asn Tyr Ser Asp Gln Leu Ala Met Ser Gly
            180                 185                 190
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Tyr Ser Met Met Lys Ser Gly Leu Pro Gly His Glu Thr Ala Met Ser
        195                 200                 205

Glu Thr Lys Lys Glu Ile Arg Ser Leu Lys Gly Ser Val Leu Ser Val
    210                 215                 220

Arg
225


<210> SEQ ID NO 16
<211> LENGTH: 324
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(324)
<223> OTHER INFORMATION: YlPex19p; GenBank Accession No. AAK84827

<400> SEQUENCE: 16

Met Ser His Glu Glu Asp Leu Asp Asp Leu Asp Asp Phe Leu Asp Glu
1               5                   10                  15

Phe Asp Glu Gln Val Leu Ser Lys Pro Pro Gly Ala Gln Lys Asp Ala
            20                  25                  30

Thr Pro Thr Thr Ser Thr Ala Pro Thr Thr Ala Glu Ala Lys Pro Asp
        35                  40                  45

Ala Thr Lys Lys Ser Thr Glu Thr Ser Gly Thr Asp Ser Lys Thr Glu
    50                  55                  60

Gly Ala Asp Thr Ala Asp Lys Asn Ala Ala Thr Asp Ser Ala Glu Ala
65                  70                  75                  80

Gly Ala Glu Lys Val Ser Leu Pro Asn Leu Glu Asp Gln Leu Ala Gly
                85                  90                  95

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

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

Ala Thr Ala Ser Glu Lys Ala Gln Gln Pro Lys Ser Phe Lys Glu Thr
    130                 135                 140

Ile Ser Ala Thr Ala Asp Arg Leu Asn Gln Ser Asn Gln Glu Met Gly
145                 150                 155                 160

Asp Met Pro Leu Gly Asp Asp Met Leu Ala Gly Leu Met Glu Gln Leu
                165                 170                 175

Ser Gly Ala Gly Gly Phe Gly Glu Gly Gly Glu Gly Asp Phe Gly Asp
            180                 185                 190

Met Leu Gly Gly Ile Met Arg Gln Leu Ala Ser Lys Glu Val Leu Tyr
        195                 200                 205

Gln Pro Leu Lys Glu Met His Asp Asn Tyr Pro Lys Trp Trp Asp Glu
    210                 215                 220

His Gly Ser Lys Val Thr Glu Glu Lys Glu Arg Asp Arg Leu Lys Leu
225                 230                 235                 240

Gln Gln Asp Ile Val Gly Lys Ile Cys Ala Lys Phe Glu Asp Pro Ser
                245                 250                 255

Tyr Ser Asp Asp Ser Glu Ala Asp Arg Ala Val Ile Thr Gln Leu Met
            260                 265                 270

Asp Glu Met Gln Glu Thr Gly Ala Pro Pro Asp Glu Ile Met Ser Asn
        275                 280                 285

Val Ala Asp Gly Ser Ile Pro Gly Gly Leu Asp Gly Leu Gly Leu Gly
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    290                 295                 300

Gly Leu Gly Gly Gly Lys Met Pro Glu Met Pro Glu Asn Met Pro Glu
305                 310                 315                 320

Cys Asn Gln Gln


<210> SEQ ID NO 17
<211> LENGTH: 417
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(417)
<223> OTHER INFORMATION: YlPex20p; GenBank Accession No. CAG79226

<400> SEQUENCE: 17

Met Ala Ser Cys Gly Pro Ser Asn Ala Leu Gln Asn Leu Ser Lys His
1               5                   10                  15

Ala Ser Ala Asp Arg Ser Leu Gln His Asp Arg Met Ala Pro Gly Gly
            20                  25                  30

Ala Pro Gly Ala Gln Arg Gln Gln Phe Arg Ser Gln Thr Gln Gly Gly
        35                  40                  45

Gln Leu Asn Asn Glu Phe Gln Gln Phe Ala Gln Ala Gly Pro Ala His
    50                  55                  60

Asn Ser Phe Glu Gln Ser Gln Met Gly Pro His Phe Gly Gln Gln His
65                  70                  75                  80

Phe Gly Gln Pro His Gln Pro Gln Met Gly Gln His Ala Pro Met Ala
                85                  90                  95

His Gly Gln Gln Ser Asp Trp Ala Gln Ser Phe Ser Gln Leu Asn Leu
            100                 105                 110

Gly Pro Gln Thr Gly Pro Gln His Thr Gln Gln Ser Asn Trp Gly Gln
        115                 120                 125

Asp Phe Met Arg Gln Ser Pro Gln Ser His Gln Val Gln Pro Gln Met
    130                 135                 140

Ala Asn Gly Val Met Gly Ser Met Ser Gly Met Ser Ser Phe Gly Pro
145                 150                 155                 160

Met Tyr Ser Asn Ser Gln Leu Met Asn Ser Thr Tyr Gly Leu Gln Thr
                165                 170                 175

Glu His Gln Gln Thr His Lys Thr Glu Thr Lys Ser Ser Gln Asp Ala
            180                 185                 190

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

Ser Asp Lys Gly Lys Glu Val Glu Lys Asp Pro Met Glu Gln Thr Tyr
    210                 215                 220

Arg Tyr Asp Gln Ala Asp Ala Leu Asn Arg Gln Ala Glu His Ile Ser
225                 230                 235                 240

Asp Asn Ile Ser Arg Glu Glu Val Asp Ile Lys Thr Asp Glu Asn Gly
                245                 250                 255

Glu Phe Ala Ser Ile Ala Arg Gln Ile Ala Ser Ser Leu Glu Glu Ala
            260                 265                 270

Asp Lys Ser Lys Phe Glu Lys Ser Thr Phe Met Asn Leu Met Arg Arg
        275                 280                 285

Ile Gly Asn His Glu Val Thr Leu Asp Gly Asp Lys Leu Val Asn Lys
    290                 295                 300
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```
Glu Gly Glu Asp Ile Arg Glu Glu Val Arg Asp Glu Leu Leu Arg Glu
305                 310                 315                 320

Gly Ala Ser Gln Glu Asn Gly Phe Gln Ser Glu Ala Gln Gln Thr Ala
                325                 330                 335

Pro Leu Pro Val His His Glu Ala Pro Pro Pro Glu Gln Ile His Pro
            340                 345                 350

His Thr Glu Thr Gly Asp Lys Gln Leu Glu Asp Pro Met Val Tyr Ile
        355                 360                 365

Glu Gln Glu Ala Ala Arg Arg Ala Ala Glu Ser Gly Arg Thr Val Glu
    370                 375                 380

Glu Glu Lys Leu Asn Phe Tyr Ser Pro Phe Glu Tyr Ala Gln Lys Leu
385                 390                 395                 400

Gly Pro Gln Gly Val Ala Lys Gln Ser Asn Trp Glu Glu Asp Tyr Asp
                405                 410                 415

Phe


<210> SEQ ID NO 18
<211> LENGTH: 195
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(195)
<223> OTHER INFORMATION: YlPex22p; GenBank Accession No. CAG77876

<400> SEQUENCE: 18

Val Pro Arg Cys Thr Ser His Pro Cys Asn Leu Thr Leu His Leu Pro
1               5                   10                  15

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

Ala Ala Ala Ala Ala Ala Ala Val Ala Tyr Leu Val Tyr Ser Phe Val
        35                  40                  45

Ala Lys Ser Asn Ser Asp Gln Asp Thr Phe Asp Ser Ser Val Gln Ser
    50                  55                  60

Ser Ser Lys Ser Ser Thr Lys Ser Pro Lys Ser Thr Ala Thr Asn Ser
65                  70                  75                  80

Lys Ile Thr Val Val Val Ser Gln Glu Leu Val Gln Ser Gln Leu Val
                85                  90                  95

Asp Phe Lys His Leu Met Ser Val His Pro Asn Leu Val Val Ile Val
            100                 105                 110

Pro Pro Met Val Ala Asn Lys Phe His Arg Ala Leu Lys Ser Ser Val
        115                 120                 125

Gly His Asp His Gly Val Lys Val Ile Arg Cys Asp Thr Asp Val Gly
    130                 135                 140

Val Ile His Val Ile Lys His Ile Arg Pro Asp Leu Ala Leu Ile Ala
145                 150                 155                 160

Asp Gly Val Gly Asp Asn Ile Gln Gly Glu Ile Lys Arg Phe Val Gly
                165                 170                 175

Ser Ser Glu Ala Leu Ser Gly Asp Val Asn Leu Ala Ala Glu Arg Leu
            180                 185                 190

Thr Gly Leu
        195


<210> SEQ ID NO 19
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<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(386)
<223> OTHER INFORMATION: YlPex26p; GenBank Accession No. NC_006072, antisense translation of nucleotides 117230-118387

<400> SEQUENCE: 19

```
Met Pro Pro Ala Met Pro Gln Met Thr Thr Ser Thr Leu Leu Thr Asp
1               5                  10                  15

Ser Val Thr Ser Ala Val Asn Gln Ala Ala Thr Pro Lys Val Asp Gln
            20                  25                  30

Met Tyr Gln Thr Phe Gly Glu Ser Ala Arg Glu Phe Val Asn Lys Asn
        35                  40                  45

Phe Tyr Asn Ser Tyr Glu Leu Ile Arg Pro Phe Phe Asp Glu Ile Thr
    50                  55                  60

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

Pro His Asn Ile Pro Leu Ser Leu Trp Ile Lys Val Trp Ser Leu Tyr
                85                  90                  95

Leu Ala Ile Leu Asp Ala Ser Cys Lys Gln Ala Gly Glu Ala Leu Leu
            100                 105                 110

Asn Ser Thr Gly Asp Leu Ser Gly Ser Asp Ser Gly Glu Trp Asn Gln
        115                 120                 125

Thr Arg Lys Leu Leu Ala Arg Lys Leu Thr Ser Gly Ser Val Trp Asp
    130                 135                 140

Glu Leu Val Thr Ala Ser Gly Gly Thr Gly Asn Ile His Pro Thr Ile
145                 150                 155                 160

Leu Ala Leu Leu Ala Ser Leu Ser Ile Arg His Asp Thr Asp Ala Lys
                165                 170                 175

Leu Met Ala Asp Asn Leu Glu Lys Phe Ile Val Thr Tyr Asn Asp Asn
            180                 185                 190

Gly Ser Asp Asp Val Lys Thr Lys Thr Ala Phe Tyr Lys Val Leu Asp
        195                 200                 205

Leu Tyr Leu Leu Arg Val Leu Pro Asp Leu Gly Gln Trp Asp Val Ala
    210                 215                 220

His Ser Phe Val Asn Asn Thr Asn Leu Phe Ser His Glu Gln Lys Lys
225                 230                 235                 240

Glu Met Thr His Lys Leu Asp Gln Ser Gln Lys His Ala Glu Gln Glu
                245                 250                 255

His Lys Arg Leu Leu Glu Glu Ala Gln Glu Lys Glu Lys Ser Asp Ala
            260                 265                 270

Lys Glu Lys Glu Arg Glu Glu Arg Val Ser Arg Asp Thr Gln Ser Arg
        275                 280                 285

Glu Ile Lys Ser Pro Ile Val Asp Ser Ser Thr Ser Ser Arg Asp Val
    290                 295                 300

Thr Arg Asp Thr Thr Arg Glu Leu Ser Lys Ser Ser Arg Gln Pro Arg
305                 310                 315                 320

Thr Leu Ser Gln Ile Ile Ser Thr Ser Leu Lys Ser Gln Phe Asp Gly
                325                 330                 335

Asn Ala Ile Phe Arg Thr Leu Ala Leu Ile Val Ile Val Ser Leu Ser
            340                 345                 350
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```
Ala Ala Asn Pro Leu Ile Arg Lys Arg Val Val Asp Thr Leu Lys Met
        355                 360                 365

Leu Trp Ile Lys Ile Leu Gln Thr Leu Ser Met Gly Phe Lys Val Ser
    370                 375                 380

Tyr Leu
385


<210> SEQ ID NO 20
<211> LENGTH: 3387
<212> TYPE: DNA
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(3387)
<223> OTHER INFORMATION: GenBank Accession No. AB036770

<400> SEQUENCE: 20

ggtaccatca agggtaaaat caaggctatc atcaagggcc atatatcgca agtttggggg     60

aagataatat gttcatagtg aatcgggttg tggatttcct catctaacgg cattataact    120

agtcctggag ggtctttttt atggataacc tccatgtacg atgtatccaa gatctccacg    180

tactgtgttc tgtttcctaa gtaataccca acaacctctc caacaaacac ttgggaagat    240

gcacttgtgc tgagatgtca agatgttaga gagtagagac agtagcaagc gtaaaaggcg    300

gccgaggcca ccgagagaac agcgtagcag ggcgcgtagt caccacaggg gacgcagaac    360

caaacaaatg acgaagaaga accacaagga gacgttttca aaggcaatgc aaacgaagag    420

ggcaatggaa ggattgagat tagagaactg gagactggag tggcgttttc ccgatgaacg    480

aacaaacacg cgaagctatg tggaccaaca tacaacacgg actgaaccag gtttttttat    540

gatttttta ctggaaatag gtacgtgcca agttggacca tgacactaaa cgtgtttaat    600

tagtaatatt cgtgtaagcg tacattcatt tcaaaggtta ttctttcacg gcaaagttat    660

aattaaatga atgtatatgc agaaaaaaaa aaaaaaagta ctgtactgga tggagagaat    720

attaataaat aattgttacc caactacatc ttgtcgattg aaagagaccc ctaagacaga    780

taggatatct gcaacccgag gaatgaaccc cccagcaccg gcacccttc tattaacaaa    840

atgccaactg aaatttgaaa agttcaacta aacttatttg acccacaaaa actcgtcaaa    900

agtggcggcg aaagctggca aatgatgaca tccccttgga accatgatat cctctcggaa    960

tcttcgtccc catttgccac atctacttgc aacgccacat ctgcttacta agcaacccaa   1020

atctgcctcg gctcaaaatg tggggaagtt cacatgcatt cgctggtgaa tctgatctga   1080

cactacaact acacaccagg tccaacatga gcgacaatac gacaatcaaa aagccgatcc   1140

gacccaaacc gatccggacg gaacgcctgc cttacgctgg ggccgcagaa atcatccgag   1200

ccaaccagaa agaccactac tttgagtccg tgcttgaaca gcatctcgtc acgtttctgc   1260

agaaatggaa gggagtacga tttatccacc agtacaagga ggagctggag acggcgtcca   1320

agtttgcata tctcggtttg tgtacgcttg tgggctccaa gactctcgga gaagagtaca   1380

ccaatctcat gtacactatc agagaccgaa cagctctacc gggggtggtg agacggtttg   1440

gctacgtgct ttccaacact ctgtttccat acctgtttgt gcgctacatg ggcaagttgc   1500

gcgccaaact gatgcgcgag tatccccatc tggtggagta cgacgaagat gagcctgtgc   1560

ccagcccgga aacatggaag gagcgggtca tcaagacgtt tgtgaacaag tttgacaagt   1620
```

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tcacggcgct ggaggggttt accgcgatcc acttggcgat tttctacgtc tacggctcgt   1680

actaccagct cagtaagcgg atctggggca tgcgttatgt atttggacac cgactggaca   1740

agaatgagcc tcgaatcggt tacgagatgc tcggtctgct gattttcgcc cggtttgcca   1800

cgtcatttgt gcagacggga agagagtacc tcggagcgct gctggaaaag agcgtggaga   1860

aagaggcagg ggagaaggaa gatgaaaagg aagcggttgt gccgaaaaag aagtcgtcaa   1920

ttccgttcat tgaggataca gaaggggaga cggaagacaa gatcgatctg gaggaccctc   1980

gacagctcaa gttcattcct gaggcgtcca gagcgtgcac tctgtgtctg tcatacatta   2040

gtgcgccggc atgtacgcca tgtggacact ttttctgttg ggactgtatt tccgaatggg   2100

tgagagagaa gcccgagtgt cccttgtgtc ggcagggtgt gagagagcag aacttgttgc   2160

ctatcagata atgacgaggt ctggatggaa ggactagtca gcgagacaca gagcatcagg   2220

gaccagacac gaccaattca atcgacaaca ctgtgctgca tagcagtgca cagaggtcct   2280

gggcatgaat atattttagc attggagata tgagtggtag agcgtataca gtattaattg   2340

tggaggtatc tcgtcgcatt gatagagcaa tacagttact gctgaaggga atgataccga   2400

gtatttcggc ccgattcagt tcttgatatc gtcattttgt ctctattgtc tacttttcag   2460

ataacctcaa caaatcttca acaaatctcc cagtaaacag tcagagatca tatccgagat   2520

catatcagat atgtcacgat ccgagtacaa taatggatat taatctgctt gattttgaat   2580

tctgttgcga ttatgatttc tttgatttcg atatgaacac atacggcgac tcccagacct   2640

ttagaagctc cagtttggat tcttagcaat ggttacactc aactatatcc caagtaatac   2700

ttggtaacaa tatgccaagt tagtcattca ttcgttatag gagttagcaa gtgtttgtca   2760

gctaaaaatg gttagtcggt cgattaccac ttagatcttt tcagcgtgga acttgatggt   2820

acgcttgaac cgacacttgg agtagtcggg gctgttgatg acgtagatga cgtttcgctc   2880

agggtgagga gtgcaatagt agtactcctt ggggccgtct ctcagctcaa aggttccatc   2940

ggcggcaatg tcaaagaccg agccctggag cttgtagccg tagtcgccgg tccagaacaa   3000

agcctgcagc tccagatagg cgatgggcat gtcgttaaca gagaaggtgt tgccctcgcc   3060

ctcggtgatg gtgatgggtt cgccgtcggt ggaggcggtg atcaggtcat cttggtaggt   3120

gacgggcaga gattcgaccg attgggcgtc tgatctggta taggtcagct tgtacttgtc   3180

tccgacagcc gccagagcgg tggtagcgac ggtgatgagg gagatgagtt tcatattggc   3240

ggcaagttta gcaaaagatg gcagtgggat tgagggacaa gagtgtttat atagatatag   3300

atacaacaca acgagtctga atgagacaac cgagacaacc actcccgaag cctcactaat   3360

agttactaac ggcatatttc aggtacc                                       3387
```

<210> SEQ ID NO 21
<211> LENGTH: 1134
<212> TYPE: DNA
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1134)
<223> OTHER INFORMATION: Pex10; GenBank Accession No. AB036770, nucleotides 1038-2171

<400> SEQUENCE: 21

```
atg tgg gga agt tca cat gca ttc gct ggt gaa tct gat ctg aca cta       48
Met Trp Gly Ser Ser His Ala Phe Ala Gly Glu Ser Asp Leu Thr Leu
1               5                   10                  15
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caa cta cac acc agg tcc aac atg agc gac aat acg aca atc aaa aag       96
Gln Leu His Thr Arg Ser Asn Met Ser Asp Asn Thr Thr Ile Lys Lys
            20                  25                  30

ccg atc cga ccc aaa ccg atc cgg acg gaa cgc ctg cct tac gct ggg      144
Pro Ile Arg Pro Lys Pro Ile Arg Thr Glu Arg Leu Pro Tyr Ala Gly
        35                  40                  45

gcc gca gaa atc atc cga gcc aac cag aaa gac cac tac ttt gag tcc      192
Ala Ala Glu Ile Ile Arg Ala Asn Gln Lys Asp His Tyr Phe Glu Ser
    50                  55                  60

gtg ctt gaa cag cat ctc gtc acg ttt ctg cag aaa tgg aag gga gta      240
Val Leu Glu Gln His Leu Val Thr Phe Leu Gln Lys Trp Lys Gly Val
65                  70                  75                  80

cga ttt atc cac cag tac aag gag gag ctg gag acg gcg tcc aag ttt      288
Arg Phe Ile His Gln Tyr Lys Glu Glu Leu Glu Thr Ala Ser Lys Phe
                85                  90                  95

gca tat ctc ggt ttg tgt acg ctt gtg ggc tcc aag act ctc gga gaa      336
Ala Tyr Leu Gly Leu Cys Thr Leu Val Gly Ser Lys Thr Leu Gly Glu
            100                 105                 110

gag tac acc aat ctc atg tac act atc aga gac cga aca gct cta ccg      384
Glu Tyr Thr Asn Leu Met Tyr Thr Ile Arg Asp Arg Thr Ala Leu Pro
        115                 120                 125

ggg gtg gtg aga cgg ttt ggc tac gtg ctt tcc aac act ctg ttt cca      432
Gly Val Val Arg Arg Phe Gly Tyr Val Leu Ser Asn Thr Leu Phe Pro
    130                 135                 140

tac ctg ttt gtg cgc tac atg ggc aag ttg cgc gcc aaa ctg atg cgc      480
Tyr Leu Phe Val Arg Tyr Met Gly Lys Leu Arg Ala Lys Leu Met Arg
145                 150                 155                 160

gag tat ccc cat ctg gtg gag tac gac gaa gat gag cct gtg ccc agc      528
Glu Tyr Pro His Leu Val Glu Tyr Asp Glu Asp Glu Pro Val Pro Ser
                165                 170                 175

ccg gaa aca tgg aag gag cgg gtc atc aag acg ttt gtg aac aag ttt      576
Pro Glu Thr Trp Lys Glu Arg Val Ile Lys Thr Phe Val Asn Lys Phe
            180                 185                 190

gac aag ttc acg gcg ctg gag ggg ttt acc gcg atc cac ttg gcg att      624
Asp Lys Phe Thr Ala Leu Glu Gly Phe Thr Ala Ile His Leu Ala Ile
        195                 200                 205

ttc tac gtc tac ggc tcg tac tac cag ctc agt aag cgg atc tgg ggc      672
Phe Tyr Val Tyr Gly Ser Tyr Tyr Gln Leu Ser Lys Arg Ile Trp Gly
    210                 215                 220

atg cgt tat gta ttt gga cac cga ctg gac aag aat gag cct cga atc      720
Met Arg Tyr Val Phe Gly His Arg Leu Asp Lys Asn Glu Pro Arg Ile
225                 230                 235                 240

ggt tac gag atg ctc ggt ctg ctg att ttc gcc cgg ttt gcc acg tca      768
Gly Tyr Glu Met Leu Gly Leu Leu Ile Phe Ala Arg Phe Ala Thr Ser
                245                 250                 255

ttt gtg cag acg gga aga gag tac ctc gga gcg ctg ctg gaa aag agc      816
Phe Val Gln Thr Gly Arg Glu Tyr Leu Gly Ala Leu Leu Glu Lys Ser
            260                 265                 270

gtg gag aaa gag gca ggg gag aag gaa gat gaa aag gaa gcg gtt gtg      864
Val Glu Lys Glu Ala Gly Glu Lys Glu Asp Glu Lys Glu Ala Val Val
        275                 280                 285

ccg aaa aag aag tcg tca att ccg ttc att gag gat aca gaa ggg gag      912
Pro Lys Lys Lys Ser Ser Ile Pro Phe Ile Glu Asp Thr Glu Gly Glu
    290                 295                 300

acg gaa gac aag atc gat ctg gag gac cct cga cag ctc aag ttc att      960
Thr Glu Asp Lys Ile Asp Leu Glu Asp Pro Arg Gln Leu Lys Phe Ile
305                 310                 315                 320
```

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```
cct gag gcg tcc aga gcg tgc act ctg tgt ctg tca tac att agt gcg     1008
Pro Glu Ala Ser Arg Ala Cys Thr Leu Cys Leu Ser Tyr Ile Ser Ala
                325                 330                 335

ccg gca tgt acg cca tgt gga cac ttt ttc tgt tgg gac tgt att tcc     1056
Pro Ala Cys Thr Pro Cys Gly His Phe Phe Cys Trp Asp Cys Ile Ser
            340                 345                 350

gaa tgg gtg aga gag aag ccc gag tgt ccc ttg tgt cgg cag ggt gtg     1104
Glu Trp Val Arg Glu Lys Pro Glu Cys Pro Leu Cys Arg Gln Gly Val
        355                 360                 365

aga gag cag aac ttg ttg cct atc aga taa                             1134
Arg Glu Gln Asn Leu Leu Pro Ile Arg
    370                 375


<210> SEQ ID NO 22
<211> LENGTH: 377
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica

<400> SEQUENCE: 22

Met Trp Gly Ser Ser His Ala Phe Ala Gly Glu Ser Asp Leu Thr Leu
1               5                   10                  15

Gln Leu His Thr Arg Ser Asn Met Ser Asp Asn Thr Thr Ile Lys Lys
            20                  25                  30

Pro Ile Arg Pro Lys Pro Ile Arg Thr Glu Arg Leu Pro Tyr Ala Gly
        35                  40                  45

Ala Ala Glu Ile Ile Arg Ala Asn Gln Lys Asp His Tyr Phe Glu Ser
    50                  55                  60

Val Leu Glu Gln His Leu Val Thr Phe Leu Gln Lys Trp Lys Gly Val
65                  70                  75                  80

Arg Phe Ile His Gln Tyr Lys Glu Glu Leu Glu Thr Ala Ser Lys Phe
                85                  90                  95

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

Glu Tyr Thr Asn Leu Met Tyr Thr Ile Arg Asp Arg Thr Ala Leu Pro
        115                 120                 125

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

Tyr Leu Phe Val Arg Tyr Met Gly Lys Leu Arg Ala Lys Leu Met Arg
145                 150                 155                 160

Glu Tyr Pro His Leu Val Glu Tyr Asp Glu Asp Glu Pro Val Pro Ser
                165                 170                 175

Pro Glu Thr Trp Lys Glu Arg Val Ile Lys Thr Phe Val Asn Lys Phe
            180                 185                 190

Asp Lys Phe Thr Ala Leu Glu Gly Phe Thr Ala Ile His Leu Ala Ile
        195                 200                 205

Phe Tyr Val Tyr Gly Ser Tyr Tyr Gln Leu Ser Lys Arg Ile Trp Gly
    210                 215                 220

Met Arg Tyr Val Phe Gly His Arg Leu Asp Lys Asn Glu Pro Arg Ile
225                 230                 235                 240

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

Phe Val Gln Thr Gly Arg Glu Tyr Leu Gly Ala Leu Leu Glu Lys Ser
            260                 265                 270
```

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```
Val Glu Lys Glu Ala Gly Glu Lys Glu Asp Glu Lys Glu Ala Val Val
        275                 280                 285

Pro Lys Lys Lys Ser Ser Ile Pro Phe Ile Glu Asp Thr Glu Gly Glu
    290                 295                 300

Thr Glu Asp Lys Ile Asp Leu Glu Asp Pro Arg Gln Leu Lys Phe Ile
305                 310                 315                 320

Pro Glu Ala Ser Arg Ala Cys Thr Leu Cys Leu Ser Tyr Ile Ser Ala
                325                 330                 335

Pro Ala Cys Thr Pro Cys Gly His Phe Phe Cys Trp Asp Cys Ile Ser
            340                 345                 350

Glu Trp Val Arg Glu Lys Pro Glu Cys Pro Leu Cys Arg Gln Gly Val
        355                 360                 365

Arg Glu Gln Asn Leu Leu Pro Ile Arg
    370                 375


<210> SEQ ID NO 23
<211> LENGTH: 1065
<212> TYPE: DNA
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1065)
<223> OTHER INFORMATION: YlPEX10; GenBank Accession No. AJ012084, which
      corresponds to nucleotides 1107-2171 of GenBank Accession No.
      AB036770

<400> SEQUENCE: 23

atg agc gac aat acg aca atc aaa aag ccg atc cga ccc aaa ccg atc       48
Met Ser Asp Asn Thr Thr Ile Lys Lys Pro Ile Arg Pro Lys Pro Ile
1               5                   10                  15

cgg acg gaa cgc ctg cct tac gct ggg gcc gca gaa atc atc cga gcc       96
Arg Thr Glu Arg Leu Pro Tyr Ala Gly Ala Ala Glu Ile Ile Arg Ala
            20                  25                  30

aac cag aaa gac cac tac ttt gag tcc gtg ctt gaa cag cat ctc gtc      144
Asn Gln Lys Asp His Tyr Phe Glu Ser Val Leu Glu Gln His Leu Val
        35                  40                  45

acg ttt ctg cag aaa tgg aag gga gta cga ttt atc cac cag tac aag      192
Thr Phe Leu Gln Lys Trp Lys Gly Val Arg Phe Ile His Gln Tyr Lys
    50                  55                  60

gag gag ctg gag acg gcg tcc aag ttt gca tat ctc ggt ttg tgt acg      240
Glu Glu Leu Glu Thr Ala Ser Lys Phe Ala Tyr Leu Gly Leu Cys Thr
65                  70                  75                  80

ctt gtg ggc tcc aag act ctc gga gaa gag tac acc aat ctc atg tac      288
Leu Val Gly Ser Lys Thr Leu Gly Glu Glu Tyr Thr Asn Leu Met Tyr
                85                  90                  95

act atc aga gac cga aca gct cta ccg ggg gtg gtg aga cgg ttt ggc      336
Thr Ile Arg Asp Arg Thr Ala Leu Pro Gly Val Val Arg Arg Phe Gly
            100                 105                 110

tac gtg ctt tcc aac act ctg ttt cca tac ctg ttt gtg cgc tac atg      384
Tyr Val Leu Ser Asn Thr Leu Phe Pro Tyr Leu Phe Val Arg Tyr Met
        115                 120                 125

ggc aag ttg cgc gcc aaa ctg atg cgc gag tat ccc cat ctg gtg gag      432
Gly Lys Leu Arg Ala Lys Leu Met Arg Glu Tyr Pro His Leu Val Glu
    130                 135                 140

tac gac gaa gat gag cct gtg ccc agc ccg gaa aca tgg aag gag cgg      480
Tyr Asp Glu Asp Glu Pro Val Pro Ser Pro Glu Thr Trp Lys Glu Arg
145                 150                 155                 160

gtc atc aag acg ttt gtg aac aag ttt gac aag ttc acg gcg ctg gag      528
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```
Val Ile Lys Thr Phe Val Asn Lys Phe Asp Lys Phe Thr Ala Leu Glu
                165                 170                 175

ggg ttt acc gcg atc cac ttg gcg att ttc tac gtc tac ggc tcg tac      576
Gly Phe Thr Ala Ile His Leu Ala Ile Phe Tyr Val Tyr Gly Ser Tyr
            180                 185                 190

tac cag ctc agt aag cgg atc tgg ggc atg cgt tat gta ttt gga cac      624
Tyr Gln Leu Ser Lys Arg Ile Trp Gly Met Arg Tyr Val Phe Gly His
        195                 200                 205

cga ctg gac aag aat gag cct cga atc ggt tac gag atg ctc ggt ctg      672
Arg Leu Asp Lys Asn Glu Pro Arg Ile Gly Tyr Glu Met Leu Gly Leu
    210                 215                 220

ctg att ttc gcc cgg ttt gcc acg tca ttt gtg cag acg gga aga gag      720
Leu Ile Phe Ala Arg Phe Ala Thr Ser Phe Val Gln Thr Gly Arg Glu
225                 230                 235                 240

tac ctc gga gcg ctg ctg gaa aag agc gtg gag aaa gag gca ggg gag      768
Tyr Leu Gly Ala Leu Leu Glu Lys Ser Val Glu Lys Glu Ala Gly Glu
                245                 250                 255

aag gaa gat gaa aag gaa gcg gtt gtg ccg aaa aag aag tcg tca att      816
Lys Glu Asp Glu Lys Glu Ala Val Val Pro Lys Lys Lys Ser Ser Ile
            260                 265                 270

ccg ttc att gag gat aca gaa ggg gag acg gaa gac aag atc gat ctg      864
Pro Phe Ile Glu Asp Thr Glu Gly Glu Thr Glu Asp Lys Ile Asp Leu
        275                 280                 285

gag gac cct cga cag ctc aag ttc att cct gag gcg tcc aga gcg tgc      912
Glu Asp Pro Arg Gln Leu Lys Phe Ile Pro Glu Ala Ser Arg Ala Cys
    290                 295                 300

act ctg tgt ctg tca tac att agt gcg ccg gca tgt acg cca tgt gga      960
Thr Leu Cys Leu Ser Tyr Ile Ser Ala Pro Ala Cys Thr Pro Cys Gly
305                 310                 315                 320

cac ttt ttc tgt tgg gac tgt att tcc gaa tgg gtg aga gag aag ccc     1008
His Phe Phe Cys Trp Asp Cys Ile Ser Glu Trp Val Arg Glu Lys Pro
                325                 330                 335

gag tgt ccc ttg tgt cgg cag ggt gtg aga gag cag aac ttg ttg cct     1056
Glu Cys Pro Leu Cys Arg Gln Gly Val Arg Glu Gln Asn Leu Leu Pro
            340                 345                 350

atc aga taa                                                        1065
Ile Arg
```

<210> SEQ ID NO 24
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica

<400> SEQUENCE: 24

```
Met Ser Asp Asn Thr Thr Ile Lys Lys Pro Ile Arg Pro Lys Pro Ile
1               5                  10                  15

Arg Thr Glu Arg Leu Pro Tyr Ala Gly Ala Ala Glu Ile Ile Arg Ala
            20                  25                  30

Asn Gln Lys Asp His Tyr Phe Glu Ser Val Leu Glu Gln His Leu Val
        35                  40                  45

Thr Phe Leu Gln Lys Trp Lys Gly Val Arg Phe Ile His Gln Tyr Lys
    50                  55                  60

Glu Glu Leu Glu Thr Ala Ser Lys Phe Ala Tyr Leu Gly Leu Cys Thr
65                  70                  75                  80

Leu Val Gly Ser Lys Thr Leu Gly Glu Glu Tyr Thr Asn Leu Met Tyr
                85                  90                  95
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```
Thr Ile Arg Asp Arg Thr Ala Leu Pro Gly Val Val Arg Arg Phe Gly
            100                 105                 110

Tyr Val Leu Ser Asn Thr Leu Phe Pro Tyr Leu Phe Val Arg Tyr Met
        115                 120                 125

Gly Lys Leu Arg Ala Lys Leu Met Arg Glu Tyr Pro His Leu Val Glu
    130                 135                 140

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

Val Ile Lys Thr Phe Val Asn Lys Phe Asp Lys Phe Thr Ala Leu Glu
                165                 170                 175

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

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

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

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

Tyr Leu Gly Ala Leu Leu Glu Lys Ser Val Glu Lys Glu Ala Gly Glu
                245                 250                 255

Lys Glu Asp Glu Lys Glu Ala Val Val Pro Lys Lys Lys Ser Ser Ile
            260                 265                 270

Pro Phe Ile Glu Asp Thr Glu Gly Glu Thr Glu Asp Lys Ile Asp Leu
        275                 280                 285

Glu Asp Pro Arg Gln Leu Lys Phe Ile Pro Glu Ala Ser Arg Ala Cys
    290                 295                 300

Thr Leu Cys Leu Ser Tyr Ile Ser Ala Pro Ala Cys Thr Pro Cys Gly
305                 310                 315                 320

His Phe Phe Cys Trp Asp Cys Ile Ser Glu Trp Val Arg Glu Lys Pro
                325                 330                 335

Glu Cys Pro Leu Cys Arg Gln Gly Val Arg Glu Gln Asn Leu Leu Pro
            340                 345                 350

Ile Arg


<210> SEQ ID NO 25
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5)..(15)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (19)..(20)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
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<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36)..(37)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 25

Cys Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
1 5 10 15

Xaa His Xaa Xaa Cys Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            20 25 30

Xaa Xaa Cys Xaa Xaa Cys
        35

<210> SEQ ID NO 26
<211> LENGTH: 345
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica

<400> SEQUENCE: 26

Met Trp Gly Ser Ser His Ala Phe Ala Gly Glu Ser Asp Leu Thr Leu
1 5 10 15

Gln Leu His Thr Arg Ser Asn Met Ser Asp Asn Thr Thr Ile Lys Lys
            20 25 30

Pro Ile Arg Pro Lys Pro Ile Arg Thr Glu Arg Leu Pro Tyr Ala Gly
        35 40 45

Ala Ala Glu Ile Ile Arg Ala Asn Gln Lys Asp His Tyr Phe Glu Ser
    50 55 60

Val Leu Glu Gln His Leu Val Thr Phe Leu Gln Lys Trp Lys Gly Val
65 70 75 80

Arg Phe Ile His Gln Tyr Lys Glu Glu Leu Glu Thr Ala Ser Lys Phe
                85 90 95

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

Glu Tyr Thr Asn Leu Met Tyr Thr Ile Arg Asp Arg Thr Ala Leu Pro
        115 120 125

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

Tyr Leu Phe Val Arg Tyr Met Gly Lys Leu Arg Ala Lys Leu Met Arg
145 150 155 160

Glu Tyr Pro His Leu Val Glu Tyr Asp Glu Asp Glu Pro Val Pro Ser
                165 170 175

Pro Glu Thr Trp Lys Glu Arg Val Ile Lys Thr Phe Val Asn Lys Phe
            180 185 190

Asp Lys Phe Thr Ala Leu Glu Gly Phe Thr Ala Ile His Leu Ala Ile
        195 200 205

Phe Tyr Val Tyr Gly Ser Tyr Tyr Gln Leu Ser Lys Arg Ile Trp Gly
    210 215 220

Met Arg Tyr Val Phe Gly His Arg Leu Asp Lys Asn Glu Pro Arg Ile
225 230 235 240

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

Phe Val Gln Thr Gly Arg Glu Tyr Leu Gly Ala Leu Leu Glu Lys Ser

-continued

```
            260                 265                 270

Val Glu Lys Glu Ala Gly Glu Lys Glu Asp Glu Lys Glu Ala Val Val
        275                 280                 285

Pro Lys Lys Lys Ser Ser Ile Pro Phe Ile Glu Asp Thr Glu Gly Glu
    290                 295                 300

Thr Glu Asp Lys Ile Asp Leu Glu Asp Pro Arg Gln Leu Lys Phe Ile
305                 310                 315                 320

Pro Glu Ala Ser Arg Ala Cys Thr Leu Cys Leu Ser Tyr Ile Ser Ala
                325                 330                 335

Pro Ala Cys Thr Pro Cys Gly His Phe
            340                 345


<210> SEQ ID NO 27
<211> LENGTH: 2987
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<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: 5' end is associated with a -PO4 group
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<222> LOCATION: (768)..(770)
<223> OTHER INFORMATION: initiation codon ('ATG')
<300> PUBLICATION INFORMATION:
<302> TITLE: ACYLTRANSFERASES FOR ALTERATION OF POLYUNSATURATED FATTY
      ACIDS AND OIL CONTENT IN OLEAGINOUS YEASTS
<310> PATENT DOCUMENT NUMBER: U.S. Patent 7,267,976
<311> PATENT FILING DATE: 2004-07-01
<312> PUBLICATION DATE: 2007-09-11
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(2119)

<400> SEQUENCE: 72

aaacgcaccc actgctcgtc ctccttgctc ctcgaaaccg actcctctac acacgtcaaa     60

tccgaggttg aaatcttccc cacatttggc agccaaacca gcacatccca gcaacctcgc    120

acagcgccga aatcgacctg tcgacttggc cacaaaaaaa agcaccggct ctgcaacagt    180

tctcacgacc aattacgtac aagtacgaaa tcgttcgtgg accgtgactg ataagctccc    240

acttttttctt ctaacaacag gcaacagaca agtcacacaa aacaaaagct atg act       296
                                                        Met Thr
                                                        1

atc gac tca caa tac tac aag tcg cga gac aaa aac gac acg gca ccc      344
Ile Asp Ser Gln Tyr Tyr Lys Ser Arg Asp Lys Asn Asp Thr Ala Pro
        5                  10                  15

aaa atc gcg gga atc cga tat gcc ccg cta tcg aca cca tta ctc aac      392
Lys Ile Ala Gly Ile Arg Tyr Ala Pro Leu Ser Thr Pro Leu Leu Asn
    20                  25                  30

cga tgt gag acc ttc tct ctg gtc tgg cac att ttc agc att ccc act      440
Arg Cys Glu Thr Phe Ser Leu Val Trp His Ile Phe Ser Ile Pro Thr
35                  40                  45                  50

ttc ctc aca att ttc atg cta tgc tgc gca att cca ctg ctc tgg cca      488
Phe Leu Thr Ile Phe Met Leu Cys Cys Ala Ile Pro Leu Leu Trp Pro
                55                  60                  65

ttt gtg att gcg tat gta gtg tac gct gtt aaa gac gac tcc ccg tcc      536
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Phe Val Ile Ala Tyr Val Val Tyr Ala Val Lys Asp Asp Ser Pro Ser
            70                  75                  80

aac gga gga gtg gtc aag cga tac tcg cct att tca aga aac ttc ttc      584
Asn Gly Gly Val Val Lys Arg Tyr Ser Pro Ile Ser Arg Asn Phe Phe
        85                  90                  95

atc tgg aag ctc ttt ggc cgc tac ttc ccc ata act ctg cac aag acg      632
Ile Trp Lys Leu Phe Gly Arg Tyr Phe Pro Ile Thr Leu His Lys Thr
    100                 105                 110

gtg gat ctg gag ccc acg cac aca tac tac cct ctg gac gtc cag gag      680
Val Asp Leu Glu Pro Thr His Thr Tyr Tyr Pro Leu Asp Val Gln Glu
115                 120                 125                 130

tat cac ctg att gct gag aga tac tgg ccg cag aac aag tac ctc cga      728
Tyr His Leu Ile Ala Glu Arg Tyr Trp Pro Gln Asn Lys Tyr Leu Arg
                135                 140                 145

gca atc atc tcc acc atc gag tac ttt ctg ccc gcc ttc atg aaa cgg      776
Ala Ile Ile Ser Thr Ile Glu Tyr Phe Leu Pro Ala Phe Met Lys Arg
            150                 155                 160

tct ctt tct atc aac gag cag gag cag cct gcc gag cga gat cct ctc      824
Ser Leu Ser Ile Asn Glu Gln Glu Gln Pro Ala Glu Arg Asp Pro Leu
        165                 170                 175

ctg tct ccc gtt tct ccc agc tct ccg ggt tct caa cct gac aag tgg      872
Leu Ser Pro Val Ser Pro Ser Ser Pro Gly Ser Gln Pro Asp Lys Trp
    180                 185                 190

att aac cac gac agc aga tat agc cgt gga gaa tca tct ggc tcc aac      920
Ile Asn His Asp Ser Arg Tyr Ser Arg Gly Glu Ser Ser Gly Ser Asn
195                 200                 205                 210

ggc cac gcc tcg ggc tcc gaa ctt aac ggc aac ggc aac aat ggc acc      968
Gly His Ala Ser Gly Ser Glu Leu Asn Gly Asn Gly Asn Asn Gly Thr
                215                 220                 225

act aac cga cga cct ttg tcg tcc gcc tct gct ggc tcc act gca tct     1016
Thr Asn Arg Arg Pro Leu Ser Ser Ala Ser Ala Gly Ser Thr Ala Ser
            230                 235                 240

gat tcc acg ctt ctt aac ggg tcc ctc aac tcc tac gcc aac cag atc     1064
Asp Ser Thr Leu Leu Asn Gly Ser Leu Asn Ser Tyr Ala Asn Gln Ile
        245                 250                 255

att ggc gaa aac gac cca cag ctg tcg ccc aca aaa ctc aag ccc act     1112
Ile Gly Glu Asn Asp Pro Gln Leu Ser Pro Thr Lys Leu Lys Pro Thr
    260                 265                 270

ggc aga aaa tac atc ttc ggc tac cac ccc cac ggc att atc ggc atg     1160
Gly Arg Lys Tyr Ile Phe Gly Tyr His Pro His Gly Ile Ile Gly Met
275                 280                 285                 290

gga gcc ttt ggt gga att gcc acc gag gga gct gga tgg tcc aag ctc     1208
Gly Ala Phe Gly Gly Ile Ala Thr Glu Gly Ala Gly Trp Ser Lys Leu
                295                 300                 305

ttt ccg ggc atc cct gtt tct ctt atg act ctc acc aac aac ttc cga     1256
Phe Pro Gly Ile Pro Val Ser Leu Met Thr Leu Thr Asn Asn Phe Arg
            310                 315                 320

gtg cct ctc tac aga gag tac ctc atg agt ctg gga gtc gct tct gtc     1304
Val Pro Leu Tyr Arg Glu Tyr Leu Met Ser Leu Gly Val Ala Ser Val
        325                 330                 335

tcc aag aag tcc tgc aag gcc ctc ctc aag cga aac cag tct atc tgc     1352
Ser Lys Lys Ser Cys Lys Ala Leu Leu Lys Arg Asn Gln Ser Ile Cys
    340                 345                 350

att gtc gtt ggt gga gca cag gaa agt ctt ctg gcc aga ccc ggt gtc     1400
Ile Val Val Gly Gly Ala Gln Glu Ser Leu Leu Ala Arg Pro Gly Val
355                 360                 365                 370

atg gac ctg gtg cta ctc aag cga aag ggt ttt gtt cga ctt ggt atg     1448
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Met Asp Leu Val Leu Leu Lys Arg Lys Gly Phe Val Arg Leu Gly Met
                375                 380                 385

gag gtc gga aat gtc gcc ctt gtt ccc atc atg gcc ttt ggt gag aac     1496
Glu Val Gly Asn Val Ala Leu Val Pro Ile Met Ala Phe Gly Glu Asn
            390                 395                 400

gac ctc tat gac cag gtt agc aac gac aag tcg tcc aag ctg tac cga     1544
Asp Leu Tyr Asp Gln Val Ser Asn Asp Lys Ser Ser Lys Leu Tyr Arg
        405                 410                 415

ttc cag cag ttt gtc aag aac ttc ctt gga ttc acc ctt cct ttg atg     1592
Phe Gln Gln Phe Val Lys Asn Phe Leu Gly Phe Thr Leu Pro Leu Met
    420                 425                 430

cat gcc cga ggc gtc ttc aac tac gat gtc ggt ctt gtc ccc tac agg     1640
His Ala Arg Gly Val Phe Asn Tyr Asp Val Gly Leu Val Pro Tyr Arg
435                 440                 445                 450

cga ccc gtc aac att gtg gtt ggt tcc ccc att gac ttg cct tat ctc     1688
Arg Pro Val Asn Ile Val Val Gly Ser Pro Ile Asp Leu Pro Tyr Leu
                455                 460                 465

cca cac ccc acc gac gaa gaa gtg tcc gaa tac cac gac cga tac atc     1736
Pro His Pro Thr Asp Glu Glu Val Ser Glu Tyr His Asp Arg Tyr Ile
            470                 475                 480

gcc gag ctg cag cga atc tac aac gag cac aag gat gaa tat ttc atc     1784
Ala Glu Leu Gln Arg Ile Tyr Asn Glu His Lys Asp Glu Tyr Phe Ile
        485                 490                 495

gat tgg acc gag gag ggc aaa gga gcc cca gag ttc cga atg att gag     1832
Asp Trp Thr Glu Glu Gly Lys Gly Ala Pro Glu Phe Arg Met Ile Glu
    500                 505                 510

taa ggaaaactgc ctgggttagg caaatagcta atgagtattt ttttgatggc          1885

aaccaaatgt agaaagaaaa aaaaaaaaaa agaaaaaaaa aagagaatat tatatctatg   1945

taattctatt aaaagctctg ttgagtgagc ggaataaata ctgttgaaga ggggattgtg   2005

tagagatctg tttactcaat ggcaaactca tctgggggag atccttccac tgtgggaagc   2065

tcctggatag cctttgcatc ggggttcaag aagaccattg tgaacagccc ttga         2119


<210> SEQ ID NO 73
<211> LENGTH: 514
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica

<400> SEQUENCE: 73

Met Thr Ile Asp Ser Gln Tyr Tyr Lys Ser Arg Asp Lys Asn Asp Thr
1               5                  10                  15

Ala Pro Lys Ile Ala Gly Ile Arg Tyr Ala Pro Leu Ser Thr Pro Leu
            20                  25                  30

Leu Asn Arg Cys Glu Thr Phe Ser Leu Val Trp His Ile Phe Ser Ile
        35                  40                  45

Pro Thr Phe Leu Thr Ile Phe Met Leu Cys Cys Ala Ile Pro Leu Leu
    50                  55                  60

Trp Pro Phe Val Ile Ala Tyr Val Val Tyr Ala Val Lys Asp Asp Ser
65                  70                  75                  80

Pro Ser Asn Gly Gly Val Val Lys Arg Tyr Ser Pro Ile Ser Arg Asn
                85                  90                  95

Phe Phe Ile Trp Lys Leu Phe Gly Arg Tyr Phe Pro Ile Thr Leu His
            100                 105                 110

Lys Thr Val Asp Leu Glu Pro Thr His Thr Tyr Tyr Pro Leu Asp Val
        115                 120                 125
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Gln Glu Tyr His Leu Ile Ala Glu Arg Tyr Trp Pro Gln Asn Lys Tyr
    130                 135                 140

Leu Arg Ala Ile Ile Ser Thr Ile Glu Tyr Phe Leu Pro Ala Phe Met
145                 150                 155                 160

Lys Arg Ser Leu Ser Ile Asn Glu Gln Glu Gln Pro Ala Glu Arg Asp
                165                 170                 175

Pro Leu Leu Ser Pro Val Ser Pro Ser Ser Pro Gly Ser Gln Pro Asp
            180                 185                 190

Lys Trp Ile Asn His Asp Ser Arg Tyr Ser Arg Gly Glu Ser Ser Gly
        195                 200                 205

Ser Asn Gly His Ala Ser Gly Ser Glu Leu Asn Gly Asn Gly Asn Asn
    210                 215                 220

Gly Thr Thr Asn Arg Arg Pro Leu Ser Ser Ala Ser Ala Gly Ser Thr
225                 230                 235                 240

Ala Ser Asp Ser Thr Leu Leu Asn Gly Ser Leu Asn Ser Tyr Ala Asn
                245                 250                 255

Gln Ile Ile Gly Glu Asn Asp Pro Gln Leu Ser Pro Thr Lys Leu Lys
            260                 265                 270

Pro Thr Gly Arg Lys Tyr Ile Phe Gly Tyr His Pro His Gly Ile Ile
        275                 280                 285

Gly Met Gly Ala Phe Gly Gly Ile Ala Thr Glu Gly Ala Gly Trp Ser
    290                 295                 300

Lys Leu Phe Pro Gly Ile Pro Val Ser Leu Met Thr Leu Thr Asn Asn
305                 310                 315                 320

Phe Arg Val Pro Leu Tyr Arg Glu Tyr Leu Met Ser Leu Gly Val Ala
                325                 330                 335

Ser Val Ser Lys Lys Ser Cys Lys Ala Leu Leu Lys Arg Asn Gln Ser
            340                 345                 350

Ile Cys Ile Val Val Gly Gly Ala Gln Glu Ser Leu Leu Ala Arg Pro
        355                 360                 365

Gly Val Met Asp Leu Val Leu Leu Lys Arg Lys Gly Phe Val Arg Leu
    370                 375                 380

Gly Met Glu Val Gly Asn Val Ala Leu Val Pro Ile Met Ala Phe Gly
385                 390                 395                 400

Glu Asn Asp Leu Tyr Asp Gln Val Ser Asn Asp Lys Ser Ser Lys Leu
                405                 410                 415

Tyr Arg Phe Gln Gln Phe Val Lys Asn Phe Leu Gly Phe Thr Leu Pro
            420                 425                 430

Leu Met His Ala Arg Gly Val Phe Asn Tyr Asp Val Gly Leu Val Pro
        435                 440                 445

Tyr Arg Arg Pro Val Asn Ile Val Val Gly Ser Pro Ile Asp Leu Pro
    450                 455                 460

Tyr Leu Pro His Pro Thr Asp Glu Glu Val Ser Glu Tyr His Asp Arg
465                 470                 475                 480

Tyr Ile Ala Glu Leu Gln Arg Ile Tyr Asn Glu His Lys Asp Glu Tyr
                485                 490                 495

Phe Ile Asp Trp Thr Glu Glu Gly Lys Gly Ala Pro Glu Phe Arg Met
            500                 505                 510

Ile Glu
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<210> SEQ ID NO 74
<211> LENGTH: 1434
<212> TYPE: DNA
<213> ORGANISM: Fusarium moniliforme
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1434)
<223> OTHER INFORMATION: synthetic delta-12 desaturase (codon-optimized for Yarrowia lipolytica)
<300> PUBLICATION INFORMATION:
<302> TITLE: DELTA-12 DESATURASES SUITABLE FOR ALTERING LEVELS OF POLYUNSATURATED FATTY ACIDS IN OLEAGINOUS YEAST
<310> PATENT DOCUMENT NUMBER: WO 2005/047485
<311> PATENT FILING DATE: 2004-11-12
<312> PUBLICATION DATE: 2005-05-26
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(1434)
<300> PUBLICATION INFORMATION:
<302> TITLE: DELTA-12 DESATURASES SUITABLE FOR ALTERING LEVELS OF POLYUNSATURATED FATTY ACIDS IN OLEAGINOUS YEAST
<310> PATENT DOCUMENT NUMBER: US 2005-0216975-A1
<311> PATENT FILING DATE: 2004-11-10
<312> PUBLICATION DATE: 2005-09-29
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(1434)

<400> SEQUENCE: 74

```
atg gcc tcc acc tcg gct ctg ccc aag cag aac cct gcc ctc cga cga       48
Met Ala Ser Thr Ser Ala Leu Pro Lys Gln Asn Pro Ala Leu Arg Arg
1               5                   10                  15

acc gtc act tcc acc act gtg acc gac tcg gag tct gct gcc gtc tct       96
Thr Val Thr Ser Thr Thr Val Thr Asp Ser Glu Ser Ala Ala Val Ser
            20                  25                  30

ccc tcc gat tct ccc aga cac tcg gcc tcc tct aca tcg ctg tct tcc      144
Pro Ser Asp Ser Pro Arg His Ser Ala Ser Ser Thr Ser Leu Ser Ser
        35                  40                  45

atg tcc gag gtg gac att gcc aag ccc aag tcc gag tac ggt gtc atg      192
Met Ser Glu Val Asp Ile Ala Lys Pro Lys Ser Glu Tyr Gly Val Met
    50                  55                  60

ctg gat acc tac ggc aac cag ttc gaa gtt ccc gac ttc acc atc aag      240
Leu Asp Thr Tyr Gly Asn Gln Phe Glu Val Pro Asp Phe Thr Ile Lys
65                  70                  75                  80

gac atc tac aac gct att ccc aag cac tgc ttc aag cga tct gct ctc      288
Asp Ile Tyr Asn Ala Ile Pro Lys His Cys Phe Lys Arg Ser Ala Leu
                85                  90                  95

aag gga tac ggc tac att ctt cga gac att gtc ctc ctg act acc act      336
Lys Gly Tyr Gly Tyr Ile Leu Arg Asp Ile Val Leu Leu Thr Thr Thr
            100                 105                 110

ttc agc atc tgg tac aac ttt gtg aca ccc gag tac att ccc tcc act      384
Phe Ser Ile Trp Tyr Asn Phe Val Thr Pro Glu Tyr Ile Pro Ser Thr
        115                 120                 125

cct gct cga gcc ggt ctg tgg gct gtg tac acc gtt ctt cag gga ctc      432
Pro Ala Arg Ala Gly Leu Trp Ala Val Tyr Thr Val Leu Gln Gly Leu
    130                 135                 140

ttc ggt act gga ctg tgg gtc att gcc cac gag tgt gga cat ggt gct      480
Phe Gly Thr Gly Leu Trp Val Ile Ala His Glu Cys Gly His Gly Ala
145                 150                 155                 160

ttc tcc gat tcc cga atc atc aac gac att act ggc tgg gtg ctt cac      528
Phe Ser Asp Ser Arg Ile Ile Asn Asp Ile Thr Gly Trp Val Leu His
                165                 170                 175

tct tcc ctg ctt gtt ccc tac ttc agc tgg caa atc tcc cac cgg aag      576
Ser Ser Leu Leu Val Pro Tyr Phe Ser Trp Gln Ile Ser His Arg Lys
            180                 185                 190

cat cac aag gcc act gga aac atg gag cga gac atg gtc ttc gtt cct      624
His His Lys Ala Thr Gly Asn Met Glu Arg Asp Met Val Phe Val Pro
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        195                 200                 205

cga acc cga gag cag caa gct act cga ctc ggc aag atg acc cac gaa      672
Arg Thr Arg Glu Gln Gln Ala Thr Arg Leu Gly Lys Met Thr His Glu
    210                 215                 220

ctc gcc cat ctt acc gag gaa act cct gct ttc acc ctg ctc atg ctt      720
Leu Ala His Leu Thr Glu Glu Thr Pro Ala Phe Thr Leu Leu Met Leu
225                 230                 235                 240

gtg ctt cag caa ctg gtc ggt tgg ccc aac tat ctc att acc aac gtt      768
Val Leu Gln Gln Leu Val Gly Trp Pro Asn Tyr Leu Ile Thr Asn Val
                245                 250                 255

act gga cac aac tac cat gag cgg cag cga gag ggt cga ggc aag gga      816
Thr Gly His Asn Tyr His Glu Arg Gln Arg Glu Gly Arg Gly Lys Gly
            260                 265                 270

aag cac aac ggt ctt ggc ggt gga gtt aac cat ttc gat ccc cga tct      864
Lys His Asn Gly Leu Gly Gly Gly Val Asn His Phe Asp Pro Arg Ser
        275                 280                 285

cct ctg tac gag aac agc gac gcc aag ctc atc gtg ctc tcc gac att      912
Pro Leu Tyr Glu Asn Ser Asp Ala Lys Leu Ile Val Leu Ser Asp Ile
    290                 295                 300

ggc att ggt ctt atg gcc acc gct ctg tac ttt ctc gtt cag aag ttc      960
Gly Ile Gly Leu Met Ala Thr Ala Leu Tyr Phe Leu Val Gln Lys Phe
305                 310                 315                 320

gga ttc tac aac atg gcc atc tgg tac ttc gtt ccc tac ttg tgg gtt     1008
Gly Phe Tyr Asn Met Ala Ile Trp Tyr Phe Val Pro Tyr Leu Trp Val
                325                 330                 335

aac cac tgg ctc gtc gcc att acc ttt ctg cag cac aca gat cct act     1056
Asn His Trp Leu Val Ala Ile Thr Phe Leu Gln His Thr Asp Pro Thr
            340                 345                 350

ctt ccc cac tac acc aac gac gag tgg aac ttt gtg cga ggt gcc gct     1104
Leu Pro His Tyr Thr Asn Asp Glu Trp Asn Phe Val Arg Gly Ala Ala
        355                 360                 365

gca acc atc gac cga gag atg ggc ttc att gga cgt cat ctg ctc cac     1152
Ala Thr Ile Asp Arg Glu Met Gly Phe Ile Gly Arg His Leu Leu His
    370                 375                 380

ggc att atc gag act cac gtc ctg cat cac tac gtc tct tcc att ccc     1200
Gly Ile Ile Glu Thr His Val Leu His His Tyr Val Ser Ser Ile Pro
385                 390                 395                 400

ttc tac aat gcg gac gaa gct acc gag gcc atc aaa cct atc atg ggc     1248
Phe Tyr Asn Ala Asp Glu Ala Thr Glu Ala Ile Lys Pro Ile Met Gly
                405                 410                 415

aag cac tat cga gct gat gtc cag gac ggt cct cga gga ttc att cga     1296
Lys His Tyr Arg Ala Asp Val Gln Asp Gly Pro Arg Gly Phe Ile Arg
            420                 425                 430

gcc atg tac cga tct gca cga atg tgc cag tgg gtt gaa ccc tcc gct     1344
Ala Met Tyr Arg Ser Ala Arg Met Cys Gln Trp Val Glu Pro Ser Ala
        435                 440                 445

ggt gcc gag gga gct ggc aag ggt gtc ctg ttc ttt cga aac cga aac     1392
Gly Ala Glu Gly Ala Gly Lys Gly Val Leu Phe Phe Arg Asn Arg Asn
    450                 455                 460

aat gtg ggc act cct ccc gct gtc atc aag ccc gtt gcc taa             1434
Asn Val Gly Thr Pro Pro Ala Val Ile Lys Pro Val Ala
465                 470                 475
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<210> SEQ ID NO 75
<211> LENGTH: 477
<212> TYPE: PRT
<213> ORGANISM: Fusarium moniliforme

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<400> SEQUENCE: 75

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Met Ala Ser Thr Ser Ala Leu Pro Lys Gln Asn Pro Ala Leu Arg Arg
1               5                   10                  15

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

Pro Ser Asp Ser Pro Arg His Ser Ala Ser Ser Thr Ser Leu Ser Ser
        35                  40                  45

Met Ser Glu Val Asp Ile Ala Lys Pro Lys Ser Glu Tyr Gly Val Met
    50                  55                  60

Leu Asp Thr Tyr Gly Asn Gln Phe Glu Val Pro Asp Phe Thr Ile Lys
65                  70                  75                  80

Asp Ile Tyr Asn Ala Ile Pro Lys His Cys Phe Lys Arg Ser Ala Leu
                85                  90                  95

Lys Gly Tyr Gly Tyr Ile Leu Arg Asp Ile Val Leu Leu Thr Thr Thr
            100                 105                 110

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

Pro Ala Arg Ala Gly Leu Trp Ala Val Tyr Thr Val Leu Gln Gly Leu
    130                 135                 140

Phe Gly Thr Gly Leu Trp Val Ile Ala His Glu Cys Gly His Gly Ala
145                 150                 155                 160

Phe Ser Asp Ser Arg Ile Ile Asn Asp Ile Thr Gly Trp Val Leu His
                165                 170                 175

Ser Ser Leu Leu Val Pro Tyr Phe Ser Trp Gln Ile Ser His Arg Lys
            180                 185                 190

His His Lys Ala Thr Gly Asn Met Glu Arg Asp Met Val Phe Val Pro
        195                 200                 205

Arg Thr Arg Glu Gln Gln Ala Thr Arg Leu Gly Lys Met Thr His Glu
    210                 215                 220

Leu Ala His Leu Thr Glu Glu Thr Pro Ala Phe Thr Leu Leu Met Leu
225                 230                 235                 240

Val Leu Gln Gln Leu Val Gly Trp Pro Asn Tyr Leu Ile Thr Asn Val
                245                 250                 255

Thr Gly His Asn Tyr His Glu Arg Gln Arg Glu Gly Arg Gly Lys Gly
            260                 265                 270

Lys His Asn Gly Leu Gly Gly Gly Val Asn His Phe Asp Pro Arg Ser
        275                 280                 285

Pro Leu Tyr Glu Asn Ser Asp Ala Lys Leu Ile Val Leu Ser Asp Ile
    290                 295                 300

Gly Ile Gly Leu Met Ala Thr Ala Leu Tyr Phe Leu Val Gln Lys Phe
305                 310                 315                 320

Gly Phe Tyr Asn Met Ala Ile Trp Tyr Phe Val Pro Tyr Leu Trp Val
                325                 330                 335

Asn His Trp Leu Val Ala Ile Thr Phe Leu Gln His Thr Asp Pro Thr
            340                 345                 350

Leu Pro His Tyr Thr Asn Asp Glu Trp Asn Phe Val Arg Gly Ala Ala
        355                 360                 365

Ala Thr Ile Asp Arg Glu Met Gly Phe Ile Gly Arg His Leu Leu His
    370                 375                 380

Gly Ile Ile Glu Thr His Val Leu His His Tyr Val Ser Ser Ile Pro
385                 390                 395                 400
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Phe Tyr Asn Ala Asp Glu Ala Thr Glu Ala Ile Lys Pro Ile Met Gly
                405                 410                 415

Lys His Tyr Arg Ala Asp Val Gln Asp Gly Pro Arg Gly Phe Ile Arg
            420                 425                 430

Ala Met Tyr Arg Ser Ala Arg Met Cys Gln Trp Val Glu Pro Ser Ala
        435                 440                 445

Gly Ala Glu Gly Ala Gly Lys Gly Val Leu Phe Phe Arg Asn Arg Asn
    450                 455                 460

Asn Val Gly Thr Pro Pro Ala Val Ile Lys Pro Val Ala
465                 470                 475


<210> SEQ ID NO 76
<211> LENGTH: 1272
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant EgD8M delta-8 desaturase (also
      designated as "EgD8S-23")
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(1270)
<300> PUBLICATION INFORMATION:
<302> TITLE: MUTANT DELTA-8 DESATURASE GENES ENGINEERED BY TARGETED
      MUTAGENESIS AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
<310> PATENT DOCUMENT NUMBER: WO 2008/073271
<311> PATENT FILING DATE: 2007-12-05
<312> PUBLICATION DATE: 2008-06-19
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(1272)
<300> PUBLICATION INFORMATION:
<302> TITLE: MUTANT DELTA-8 DESATURASE GENES ENGINEERED BY TARGETED
      MUTAGENESIS AND THEIR USE IN MAKING POLYUNSATURATED FATTY ACIDS
<310> PATENT DOCUMENT NUMBER: US 2008-0138868-A1
<311> PATENT FILING DATE: 2006-12-07
<312> PUBLICATION DATE: 2008-06-12
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(1272)

<400> SEQUENCE: 76

c atg gtg aag gct tct cga cag gct ctg ccc ctc gtc atc gac gga aag     49
  Met Val Lys Ala Ser Arg Gln Ala Leu Pro Leu Val Ile Asp Gly Lys
  1               5                  10                  15

gtg tac gac gtc tcc gct tgg gtg aac ttc cac cct ggt gga gct gaa       97
Val Tyr Asp Val Ser Ala Trp Val Asn Phe His Pro Gly Gly Ala Glu
            20                  25                  30

atc att gag aac tac cag gga cga gat gct act gac gcc ttc atg gtt      145
Ile Ile Glu Asn Tyr Gln Gly Arg Asp Ala Thr Asp Ala Phe Met Val
        35                  40                  45

atg cac tct cag gaa gcc ttc gac aag ctc aag cga atg ccc aag atc      193
Met His Ser Gln Glu Ala Phe Asp Lys Leu Lys Arg Met Pro Lys Ile
    50                  55                  60

aac cag gct tcc gag ctg cct ccc cag gct gcc gtc aac gaa gct cag      241
Asn Gln Ala Ser Glu Leu Pro Pro Gln Ala Ala Val Asn Glu Ala Gln
65                  70                  75                  80

gag gat ttc cga aag ctc cga gaa gag ctg atc gcc act ggc atg ttt      289
Glu Asp Phe Arg Lys Leu Arg Glu Glu Leu Ile Ala Thr Gly Met Phe
                85                  90                  95

gac gcc tct ccc ctc tgg tac tcg tac aag atc ttg acc acc ctg ggt      337
Asp Ala Ser Pro Leu Trp Tyr Ser Tyr Lys Ile Leu Thr Thr Leu Gly
            100                 105                 110

ctt ggc gtg ctt gcc ttc ttc atg ctg gtc cag tac cac ctg tac ttc      385
Leu Gly Val Leu Ala Phe Phe Met Leu Val Gln Tyr His Leu Tyr Phe
        115                 120                 125
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att ggt gct ctc gtg ctc ggt atg cac tac cag caa atg gga tgg ctg      433
Ile Gly Ala Leu Val Leu Gly Met His Tyr Gln Gln Met Gly Trp Leu
    130                 135                 140

tct cat gac atc tgc cac cac cag acc ttc aag aac cga aac tgg aat      481
Ser His Asp Ile Cys His His Gln Thr Phe Lys Asn Arg Asn Trp Asn
145                 150                 155                 160

aac gtc ctg ggt ctg gtc ttt ggc aac gga ctc cag ggc ttc tcc gtg      529
Asn Val Leu Gly Leu Val Phe Gly Asn Gly Leu Gln Gly Phe Ser Val
                165                 170                 175

acc tgg tgg aag gac aga cac aac gcc cat cat tct gct acc aac gtt      577
Thr Trp Trp Lys Asp Arg His Asn Ala His His Ser Ala Thr Asn Val
            180                 185                 190

cag ggt cac gat ccc gac att gat aac ctg cct ctg ctc gcc tgg tcc      625
Gln Gly His Asp Pro Asp Ile Asp Asn Leu Pro Leu Leu Ala Trp Ser
        195                 200                 205

gag gac gat gtc act cga gct tct ccc atc tcc cga aag ctc att cag      673
Glu Asp Asp Val Thr Arg Ala Ser Pro Ile Ser Arg Lys Leu Ile Gln
    210                 215                 220

ttc caa cag tac tat ttc ctg gtc atc tgt att ctc ctg cga ttc atc      721
Phe Gln Gln Tyr Tyr Phe Leu Val Ile Cys Ile Leu Leu Arg Phe Ile
225                 230                 235                 240

tgg tgt ttc cag tct gtg ctg acc gtt cga tcc ctc aag gac cga gac      769
Trp Cys Phe Gln Ser Val Leu Thr Val Arg Ser Leu Lys Asp Arg Asp
                245                 250                 255

aac cag ttc tac cga tct cag tac aag aaa gag gcc att gga ctc gct      817
Asn Gln Phe Tyr Arg Ser Gln Tyr Lys Lys Glu Ala Ile Gly Leu Ala
            260                 265                 270

ctg cac tgg act ctc aag acc ctg ttc cac ctc ttc ttt atg ccc tcc      865
Leu His Trp Thr Leu Lys Thr Leu Phe His Leu Phe Phe Met Pro Ser
        275                 280                 285

atc ctg acc tcg atg ctg gtg ttc ttt gtt tcc gag ctc gtc ggt ggc      913
Ile Leu Thr Ser Met Leu Val Phe Phe Val Ser Glu Leu Val Gly Gly
    290                 295                 300

ttc gga att gcc atc gtg gtc ttc atg aac cac tac cct ctg gag aag      961
Phe Gly Ile Ala Ile Val Val Phe Met Asn His Tyr Pro Leu Glu Lys
305                 310                 315                 320

atc ggt gat tcc gtc tgg gac gga cat ggc ttc tct gtg ggt cag atc     1009
Ile Gly Asp Ser Val Trp Asp Gly His Gly Phe Ser Val Gly Gln Ile
                325                 330                 335

cat gag acc atg aac att cga cga ggc atc att act gac tgg ttc ttt     1057
His Glu Thr Met Asn Ile Arg Arg Gly Ile Ile Thr Asp Trp Phe Phe
            340                 345                 350

gga ggc ctg aac tac cag atc gag cac cat ctc tgg ccc acc ctg cct     1105
Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu Trp Pro Thr Leu Pro
        355                 360                 365

cga cac aac ctc act gcc gtt tcc tac cag gtg gaa cag ctg tgc cag     1153
Arg His Asn Leu Thr Ala Val Ser Tyr Gln Val Glu Gln Leu Cys Gln
    370                 375                 380

aag cac aac ctc ccc tac cga aac cct ctg ccc cat gaa ggt ctc gtc     1201
Lys His Asn Leu Pro Tyr Arg Asn Pro Leu Pro His Glu Gly Leu Val
385                 390                 395                 400

atc ctg ctc cga tac ctg tcc cag ttc gct cga atg gcc gag aag cag     1249
Ile Leu Leu Arg Tyr Leu Ser Gln Phe Ala Arg Met Ala Glu Lys Gln
                405                 410                 415

ccc ggt gcc aag gct cag taa gc                                     1272
Pro Gly Ala Lys Ala Gln
            420
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-continued

<210> SEQ ID NO 77
<211> LENGTH: 422
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 77

Met Val Lys Ala Ser Arg Gln Ala Leu Pro Leu Val Ile Asp Gly Lys
1 5 10 15

Val Tyr Asp Val Ser Ala Trp Val Asn Phe His Pro Gly Gly Ala Glu
            20 25 30

Ile Ile Glu Asn Tyr Gln Gly Arg Asp Ala Thr Asp Ala Phe Met Val
        35 40 45

Met His Ser Gln Glu Ala Phe Asp Lys Leu Lys Arg Met Pro Lys Ile
    50 55 60

Asn Gln Ala Ser Glu Leu Pro Pro Gln Ala Ala Val Asn Glu Ala Gln
65 70 75 80

Glu Asp Phe Arg Lys Leu Arg Glu Glu Leu Ile Ala Thr Gly Met Phe
                85 90 95

Asp Ala Ser Pro Leu Trp Tyr Ser Tyr Lys Ile Leu Thr Thr Leu Gly
            100 105 110

Leu Gly Val Leu Ala Phe Phe Met Leu Val Gln Tyr His Leu Tyr Phe
        115 120 125

Ile Gly Ala Leu Val Leu Gly Met His Tyr Gln Gln Met Gly Trp Leu
    130 135 140

Ser His Asp Ile Cys His His Gln Thr Phe Lys Asn Arg Asn Trp Asn
145 150 155 160

Asn Val Leu Gly Leu Val Phe Gly Asn Gly Leu Gln Gly Phe Ser Val
                165 170 175

Thr Trp Trp Lys Asp Arg His Asn Ala His His Ser Ala Thr Asn Val
            180 185 190

Gln Gly His Asp Pro Asp Ile Asp Asn Leu Pro Leu Leu Ala Trp Ser
        195 200 205

Glu Asp Asp Val Thr Arg Ala Ser Pro Ile Ser Arg Lys Leu Ile Gln
    210 215 220

Phe Gln Gln Tyr Tyr Phe Leu Val Ile Cys Ile Leu Leu Arg Phe Ile
225 230 235 240

Trp Cys Phe Gln Ser Val Leu Thr Val Arg Ser Leu Lys Asp Arg Asp
                245 250 255

Asn Gln Phe Tyr Arg Ser Gln Tyr Lys Lys Glu Ala Ile Gly Leu Ala
            260 265 270

Leu His Trp Thr Leu Lys Thr Leu Phe His Leu Phe Phe Met Pro Ser
        275 280 285

Ile Leu Thr Ser Met Leu Val Phe Phe Val Ser Glu Leu Val Gly Gly
    290 295 300

Phe Gly Ile Ala Ile Val Val Phe Met Asn His Tyr Pro Leu Glu Lys
305 310 315 320

Ile Gly Asp Ser Val Trp Asp Gly His Gly Phe Ser Val Gly Gln Ile
                325 330 335

His Glu Thr Met Asn Ile Arg Arg Gly Ile Ile Thr Asp Trp Phe Phe
            340 345 350

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Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu Trp Pro Thr Leu Pro
        355                 360                 365

Arg His Asn Leu Thr Ala Val Ser Tyr Gln Val Glu Gln Leu Cys Gln
    370                 375                 380

Lys His Asn Leu Pro Tyr Arg Asn Pro Leu Pro His Glu Gly Leu Val
385                 390                 395                 400

Ile Leu Leu Arg Tyr Leu Ser Gln Phe Ala Arg Met Ala Glu Lys Gln
                405                 410                 415

Pro Gly Ala Lys Ala Gln
            420


<210> SEQ ID NO 78
<211> LENGTH: 792
<212> TYPE: DNA
<213> ORGANISM: Eutreptiella sp. CCMP389
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(792)
<223> OTHER INFORMATION: synthetic delta-9 elongase (codon-optimized for
      Yarrowia lipolytica)
<300> PUBLICATION INFORMATION:
<302> TITLE: DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED
      FATTY ACIDS
<310> PATENT DOCUMENT NUMBER: WO 2007/061742
<311> PATENT FILING DATE: 2006-11-16
<312> PUBLICATION DATE: 2007-05-31
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(792)
<300> PUBLICATION INFORMATION:
<302> TITLE: DELTA-9 ELONGASES AND THEIR USE IN MAKING POLYUNSATURATED
      FATTY ACIDS
<310> PATENT DOCUMENT NUMBER: US 2007-0117190-A1
<311> PATENT FILING DATE: 2006-11-16
<312> PUBLICATION DATE: 2007-05-24
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(792)

<400> SEQUENCE: 78

atg gct gcc gtc atc gag gtg gcc aac gag ttc gtc gct atc act gcc       48
Met Ala Ala Val Ile Glu Val Ala Asn Glu Phe Val Ala Ile Thr Ala
1               5                   10                  15

gag acc ctt ccc aag gtg gac tat cag cga ctc tgg cga gac atc tac       96
Glu Thr Leu Pro Lys Val Asp Tyr Gln Arg Leu Trp Arg Asp Ile Tyr
            20                  25                  30

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

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

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

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

tgc gac aga gct ttc gac aac agc ttg ttc cga ttt gcc acc aag gtc      336
Cys Asp Arg Ala Phe Asp Asn Ser Leu Phe Arg Phe Ala Thr Lys Val
            100                 105                 110

ttc tac tat tcc aag ttt ctg gag tac atc gac tct ttc tac ctt ccc      384
Phe Tyr Tyr Ser Lys Phe Leu Glu Tyr Ile Asp Ser Phe Tyr Leu Pro
        115                 120                 125

ctc atg gcc aag cct ctg tcc ttt ctg cag ttc ttt cat cac ttg gga      432
Leu Met Ala Lys Pro Leu Ser Phe Leu Gln Phe Phe His His Leu Gly
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    130                 135                 140

gct cct atg gac atg tgg ctc ttc gtg cag tac tct ggc gaa tcc att      480
Ala Pro Met Asp Met Trp Leu Phe Val Gln Tyr Ser Gly Glu Ser Ile
145                 150                 155                 160

tgg atc ttt gtg ttc ctg aac gga ttc att cac ttt gtc atg tac ggc      528
Trp Ile Phe Val Phe Leu Asn Gly Phe Ile His Phe Val Met Tyr Gly
                165                 170                 175

tac tat tgg aca cgg ctg atg aag ttc aac ttt ccc atg ccc aag cag      576
Tyr Tyr Trp Thr Arg Leu Met Lys Phe Asn Phe Pro Met Pro Lys Gln
            180                 185                 190

ctc att acc gca atg cag atc acc cag ttc aac gtt ggc ttc tac ctc      624
Leu Ile Thr Ala Met Gln Ile Thr Gln Phe Asn Val Gly Phe Tyr Leu
        195                 200                 205

gtg tgg tgg tac aag gac att ccc tgt tac cga aag gat ccc atg cga      672
Val Trp Trp Tyr Lys Asp Ile Pro Cys Tyr Arg Lys Asp Pro Met Arg
    210                 215                 220

atg ctg gcc tgg atc ttc aac tac tgg tac gtc ggt acc gtt ctt ctg      720
Met Leu Ala Trp Ile Phe Asn Tyr Trp Tyr Val Gly Thr Val Leu Leu
225                 230                 235                 240

ctc ttc atc aac ttc ttt gtc aag tcc tac gtg ttt ccc aag cct aag      768
Leu Phe Ile Asn Phe Phe Val Lys Ser Tyr Val Phe Pro Lys Pro Lys
                245                 250                 255

act gcc gac aaa aag gtc cag tag                                     792
Thr Ala Asp Lys Lys Val Gln
            260
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<210> SEQ ID NO 79
<211> LENGTH: 263
<212> TYPE: PRT
<213> ORGANISM: Eutreptiella sp. CCMP389

<400> SEQUENCE: 79

```
Met Ala Ala Val Ile Glu Val Ala Asn Glu Phe Val Ala Ile Thr Ala
1               5                   10                  15

Glu Thr Leu Pro Lys Val Asp Tyr Gln Arg Leu Trp Arg Asp Ile Tyr
            20                  25                  30

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Thr Ala Asp Lys Lys Val Gln
            260


<210> SEQ ID NO 80
<211> LENGTH: 1350
<212> TYPE: DNA
<213> ORGANISM: Euglena gracilis
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1350)
<223> OTHER INFORMATION: synthetic delta-5 desaturase (codon-optimized
      for Yarrowia lipolytica)

<400> SEQUENCE: 80

atg gct ctc tcc ctt act acc gag cag ctg ctc gag cga ccc gac ctg       48
Met Ala Leu Ser Leu Thr Thr Glu Gln Leu Leu Glu Arg Pro Asp Leu
1               5                   10                  15

gtt gcc atc gac ggc att ctc tac gat ctg gaa ggt ctt gcc aag gtc       96
Val Ala Ile Asp Gly Ile Leu Tyr Asp Leu Glu Gly Leu Ala Lys Val
            20                  25                  30

cat ccc gga ggc gac ttg atc ctc gct tct ggt gcc tcc gat gct tct      144
His Pro Gly Gly Asp Leu Ile Leu Ala Ser Gly Ala Ser Asp Ala Ser
        35                  40                  45

cct ctg ttc tac tcc atg cac cct tac gtc aag ccc gag aac tcg aag      192
Pro Leu Phe Tyr Ser Met His Pro Tyr Val Lys Pro Glu Asn Ser Lys
    50                  55                  60

ctg ctt caa cag ttc gtg cga ggc aag cac gac cga acc tcc aag gac      240
Leu Leu Gln Gln Phe Val Arg Gly Lys His Asp Arg Thr Ser Lys Asp
65                  70                  75                  80

att gtc tac acc tac gac tct ccc ttt gca cag gac gtc aag cga act      288
Ile Val Tyr Thr Tyr Asp Ser Pro Phe Ala Gln Asp Val Lys Arg Thr
                85                  90                  95

atg cga gag gtc atg aaa ggt cgg aac tgg tat gcc aca cct gga ttc      336
Met Arg Glu Val Met Lys Gly Arg Asn Trp Tyr Ala Thr Pro Gly Phe
            100                 105                 110

tgg ctg cga acc gtt ggc atc att gct gtc acc gcc ttt tgc gag tgg      384
Trp Leu Arg Thr Val Gly Ile Ile Ala Val Thr Ala Phe Cys Glu Trp
        115                 120                 125

cac tgg gct act acc gga atg gtg ctg tgg ggt ctc ttg act gga ttc      432
His Trp Ala Thr Thr Gly Met Val Leu Trp Gly Leu Leu Thr Gly Phe
    130                 135                 140

atg cac atg cag atc ggc ctg tcc att cag cac gat gcc tct cat ggt      480
Met His Met Gln Ile Gly Leu Ser Ile Gln His Asp Ala Ser His Gly
145                 150                 155                 160

gcc atc agc aaa aag ccc tgg gtc aac gct ctc ttt gcc tac ggc atc      528
Ala Ile Ser Lys Lys Pro Trp Val Asn Ala Leu Phe Ala Tyr Gly Ile
                165                 170                 175

gac gtc att gga tcg tcc aga tgg atc tgg ctg cag tct cac atc atg      576
Asp Val Ile Gly Ser Ser Arg Trp Ile Trp Leu Gln Ser His Ile Met
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            180                 185                 190

cga cat cac acc tac acc aat cag cat ggt ctc gac ctg gat gcc gag      624
Arg His His Thr Tyr Thr Asn Gln His Gly Leu Asp Leu Asp Ala Glu
        195                 200                 205

tcc gca gaa cca ttc ctt gtg ttc cac aac tac cct gct gcc aac act      672
Ser Ala Glu Pro Phe Leu Val Phe His Asn Tyr Pro Ala Ala Asn Thr
    210                 215                 220

gct cga aag tgg ttt cac cga ttc cag gcc tgg tac atg tac ctc gtg      720
Ala Arg Lys Trp Phe His Arg Phe Gln Ala Trp Tyr Met Tyr Leu Val
225                 230                 235                 240

ctt gga gcc tac ggc gtt tcg ctg gtg tac aac cct ctc tac atc ttc      768
Leu Gly Ala Tyr Gly Val Ser Leu Val Tyr Asn Pro Leu Tyr Ile Phe
                245                 250                 255

cga atg cag cac aac gac acc att ccc gag tct gtc aca gcc atg cga      816
Arg Met Gln His Asn Asp Thr Ile Pro Glu Ser Val Thr Ala Met Arg
            260                 265                 270

gag aac ggc ttt ctg cga cgg tac cga acc ctt gca ttc gtt atg cga      864
Glu Asn Gly Phe Leu Arg Arg Tyr Arg Thr Leu Ala Phe Val Met Arg
        275                 280                 285

gct ttc ttc atc ttt cga acc gcc ttc ttg ccc tgg tat ctc act gga      912
Ala Phe Phe Ile Phe Arg Thr Ala Phe Leu Pro Trp Tyr Leu Thr Gly
    290                 295                 300

acc tcc ctg ctc atc acc att cct ctg gtg ccc act gct acc ggt gcc      960
Thr Ser Leu Leu Ile Thr Ile Pro Leu Val Pro Thr Ala Thr Gly Ala
305                 310                 315                 320

ttc ctc acc ttc ttt ttc atc ttg tct cac aac ttc gat ggc tcg gag     1008
Phe Leu Thr Phe Phe Phe Ile Leu Ser His Asn Phe Asp Gly Ser Glu
                325                 330                 335

cga atc ccc gac aag aac tgc aag gtc aag agc tcc gag aag gac gtt     1056
Arg Ile Pro Asp Lys Asn Cys Lys Val Lys Ser Ser Glu Lys Asp Val
            340                 345                 350

gaa gcc gat cag atc gac tgg tac aga gct cag gtg gag acc tct tcc     1104
Glu Ala Asp Gln Ile Asp Trp Tyr Arg Ala Gln Val Glu Thr Ser Ser
        355                 360                 365

acc tac ggt gga ccc att gcc atg ttc ttt act ggc ggt ctc aac ttc     1152
Thr Tyr Gly Gly Pro Ile Ala Met Phe Phe Thr Gly Gly Leu Asn Phe
    370                 375                 380

cag atc gag cat cac ctc ttt cct cga atg tcg tct tgg cac tat ccc     1200
Gln Ile Glu His His Leu Phe Pro Arg Met Ser Ser Trp His Tyr Pro
385                 390                 395                 400

ttc gtg cag caa gct gtc cga gag tgt tgc gaa cga cac gga gtt cgg     1248
Phe Val Gln Gln Ala Val Arg Glu Cys Cys Glu Arg His Gly Val Arg
                405                 410                 415

tac gtc ttc tac cct acc att gtg ggc aac atc att tcc acc ctc aag     1296
Tyr Val Phe Tyr Pro Thr Ile Val Gly Asn Ile Ile Ser Thr Leu Lys
            420                 425                 430

tac atg cac aaa gtc ggt gtg gtt cac tgt gtc aag gac gct cag gat     1344
Tyr Met His Lys Val Gly Val Val His Cys Val Lys Asp Ala Gln Asp
        435                 440                 445

tcc taa                                                           1350
Ser
```

<210> SEQ ID NO 81
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Euglena gracilis

<400> SEQUENCE: 81

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```
Met Ala Leu Ser Leu Thr Thr Glu Gln Leu Leu Glu Arg Pro Asp Leu
1               5                  10                  15

Val Ala Ile Asp Gly Ile Leu Tyr Asp Leu Glu Gly Leu Ala Lys Val
            20                  25                  30

His Pro Gly Gly Asp Leu Ile Leu Ala Ser Gly Ala Ser Asp Ala Ser
        35                  40                  45

Pro Leu Phe Tyr Ser Met His Pro Tyr Val Lys Pro Glu Asn Ser Lys
    50                  55                  60

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

Ile Val Tyr Thr Tyr Asp Ser Pro Phe Ala Gln Asp Val Lys Arg Thr
                85                  90                  95

Met Arg Glu Val Met Lys Gly Arg Asn Trp Tyr Ala Thr Pro Gly Phe
            100                 105                 110

Trp Leu Arg Thr Val Gly Ile Ile Ala Val Thr Ala Phe Cys Glu Trp
        115                 120                 125

His Trp Ala Thr Thr Gly Met Val Leu Trp Gly Leu Leu Thr Gly Phe
    130                 135                 140

Met His Met Gln Ile Gly Leu Ser Ile Gln His Asp Ala Ser His Gly
145                 150                 155                 160

Ala Ile Ser Lys Lys Pro Trp Val Asn Ala Leu Phe Ala Tyr Gly Ile
                165                 170                 175

Asp Val Ile Gly Ser Ser Arg Trp Ile Trp Leu Gln Ser His Ile Met
            180                 185                 190

Arg His His Thr Tyr Thr Asn Gln His Gly Leu Asp Leu Asp Ala Glu
        195                 200                 205

Ser Ala Glu Pro Phe Leu Val Phe His Asn Tyr Pro Ala Ala Asn Thr
    210                 215                 220

Ala Arg Lys Trp Phe His Arg Phe Gln Ala Trp Tyr Met Tyr Leu Val
225                 230                 235                 240

Leu Gly Ala Tyr Gly Val Ser Leu Val Tyr Asn Pro Leu Tyr Ile Phe
                245                 250                 255

Arg Met Gln His Asn Asp Thr Ile Pro Glu Ser Val Thr Ala Met Arg
            260                 265                 270

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

Ala Phe Phe Ile Phe Arg Thr Ala Phe Leu Pro Trp Tyr Leu Thr Gly
    290                 295                 300

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

Phe Leu Thr Phe Phe Phe Ile Leu Ser His Asn Phe Asp Gly Ser Glu
                325                 330                 335

Arg Ile Pro Asp Lys Asn Cys Lys Val Lys Ser Ser Glu Lys Asp Val
            340                 345                 350

Glu Ala Asp Gln Ile Asp Trp Tyr Arg Ala Gln Val Glu Thr Ser Ser
        355                 360                 365

Thr Tyr Gly Gly Pro Ile Ala Met Phe Phe Thr Gly Gly Leu Asn Phe
    370                 375                 380

Gln Ile Glu His His Leu Phe Pro Arg Met Ser Ser Trp His Tyr Pro
385                 390                 395                 400
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Phe Val Gln Gln Ala Val Arg Glu Cys Cys Glu Arg His Gly Val Arg
405 410 415

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

Tyr Met His Lys Val Gly Val Val His Cys Val Lys Asp Ala Gln Asp
435 440 445

Ser

<210> SEQ ID NO 82
<211> LENGTH: 6356
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Plasmid pY157

<400> SEQUENCE: 82

ttgagaagcc cattgtatat tattaggatc gtagcattat tgtggcaaaa aatattcaag 60

tgctcatgtg aattgacacg atcacgtaaa tacctggtga aattgctagt attcgtgatg 120

ttctaataca actctgttca atatttccgg cgctctcttg tatacaagag cacaagacat 180

gcaccccaca ttaaccgagg tcaagtgttt atgtatgaaa agtgacataa atcgtccaaa 240

aaaaagtagc acatagttgt atggctgtaa gttatgtgat tgtcagttct tcggccttcc 300

aactcctatg caccgtcttc aatcatctac ccccgtgccc cacaccccgc actattagag 360

tttatcacag tcagctaaac tgcttgcaca tctacacctc tgactacacc accatggatt 420

tcttcagacg gcaccagaaa aaggtgctgg cactggtagg tgtggcgctg agttcctacc 480

tgtttatcga ctatgtgaag aaaaagttct tcgagatcca gggtcgtttg agctcggagc 540

gaaccgctaa acagaatctc cggcgccgat ttgaacagaa ccagcaggat gcagatttta 600

caatcatggc tctgctatcc agcttgacga caccggtaat ggagcgttac cccgtcgacc 660

agatcaaggc agagttacag agcaagagac gccccacaga ccgggttttg gctctcgaga 720

gctccacctc gtcctcagct accgcacaaa ccgtgcccac catgacaagt ggcgccacag 780

aggagggcga gaagttaatt aactttggcc ggcctttacc tgcaggataa cttcgtataa 840

tgtatgctat acgaagttat gaattctctc tcttgagctt ttccataaca agttcttctg 900

cctccaggaa gtccatgggt ggtttgatca tggttttggt gtagtggtag tgcagtggtg 960

gtattgtgac tggggatgta gttgagaata agtcatacac aagtcagctt tcttcgagcc 1020

tcatataagt ataagtagtt caacgtatta gcactgtacc cagcatctcc gtatcgagaa 1080

acacaacaac atgccccatt ggacagatca tgcggataca caggttgtgc agtatcatac 1140

atactcgatc agacaggtcg tctgaccatc atacaagctg aacaagcgct ccatacttgc 1200

acgctctcta tatacacagt taaattacat atccatagtc taacctctaa cagttaatct 1260

tctggtaagc ctcccagcca gccttctggt atcgcttggc ctcctcaata ggatctcggt 1320

tctggccgta cagacctcgg ccgacaatta tgatatccgt tccggtagac atgacatcct 1380

caacagttcg gtactgctgt ccgagagcgt ctcccttgtc gtcaagaccc accccggggg 1440

tcagaataag ccagtcctca gagtcgccct taggtcggtt ctgggcaatg aagccaacca 1500

caaactcggg gtcggatcgg gcaagctcaa tggtctgctt ggagtactcg ccagtggcca 1560

gagagccctt gcaagacagc tcggccagca tgagcagacc tctggccagc ttctcgttgg 1620

gagaggggac taggaactcc ttgtactggg agttctcgta gtcagagacg tcctccttct 1680

-continued

tctgttcaga gacagtttcc tcggcaccag ctcgcaggcc agcaatgatt ccggttccgg 1740

gtacaccgtg ggcgttggtg atatcggacc actcggcgat tcggtgacac cggtactggt 1800

gcttgacagt gttgccaata tctgcgaact ttctgtcctc gaacaggaag aaaccgtgct 1860

taagagcaag ttccttgagg gggagcacag tgccggcgta ggtgaagtcg tcaatgatgt 1920

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What is claimed is:

1. A method of increasing the weight percent of at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids in an oleaginous eukaryotic organism having a total lipid content, a total lipid fraction and an oil fraction, comprising:

a) providing an oleaginous eukaryotic organism comprising:

1) genes encoding a functional polyunsaturated fatty acid biosynthetic pathway; and

2) a disruption in a native gene encoding a peroxisome biogenesis factor protein, thereby providing a PEX-disrupted organism, and

b) growing the PEX-disrupted organism under conditions as to increase the weight percent of at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids in the total lipid fraction or in the oil fraction, when compared to the weight percent of the at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids in the total lipid fraction or in the oil fraction in the oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

2. The method of claim **1**, wherein the increase in the weight percent of the at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids is at least 1.3 fold, when compared to the weight percent of polyunsaturated fatty acids relative to the weight percent of total fatty acids in the total lipid fraction or in the oil fraction in an oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

3. The method of claim **1**, wherein the at least one polyunsaturated fatty acid is selected from the group consisting of:

linoleic acid, conjugated linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, docosatetraenoic acid, ω-6 docosapentaenoic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, ω-3 docosapentaenoic acid, eicosadienoic acid, eicosatrienoic acid, docosahexaenoic acid, hydroxylated or epoxy fatty acids of these, C_{18} polyunsaturated fatty acids, C_{20} polyunsaturated fatty acids, and C_{22} polyunsaturated fatty acids.

4. The method of claim **1**, wherein the at least one polyunsaturated fatty acid consists of a combination of polyunsaturated fatty acids and wherein the weight percent of the combination is increased relative to the weight percent of total fatty acids.

5. The method of claim **4**, wherein the combination of polyunsaturated fatty acids consists of any combination of two or more polyunsaturated fatty acids selected from the group consisting of:

linoleic acid, conjugated linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, docosatetraenoic acid, ω-6 docosapentaenoic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, ω-3 docosapentaenoic acid, eicosadienoic acid, eicosatrienoic acid, docosahexaenoic acid, hydroxylated or epoxy fatty acids of these, a combination of C_{20} polyunsaturated fatty acids, a combination of C_{20-22} polyunsaturated fatty acids, and a combination of C_{22} polyunsaturated fatty acids.

6. The method of claim **1**, wherein the total lipid content in the PEX-disrupted organism is increased, when compared with the total lipid content in an oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

7. The method of claim **1**, wherein the total lipid content in the PEX-disrupted organism is decreased, when compared with the total lipid content in an oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

8. The method of claim **1**, wherein the PEX-disrupted organism is selected from the group consisting of: *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon, Lipomyces, Mortierella Thraustochytrium, Schizochytrium*, and *Saccharomyces* having the property of oleaginy.

9. The method of claim **1**, wherein the polyunsaturated fatty acid biosynthetic pathway comprises genes encoding enzymes selected from the group consisting of:

Δ9 desaturase, Δ12 desaturase, Δ6 desaturase, Δ5 desaturase, Δ17 desaturase, Δ8 desaturase, Δ15 desaturase, Δ4 desaturase, $C_{14/16}$ elongase, $C_{16/18}$ elongase, $C_{18/20}$ elongase, $C_{20/22}$ elongase and Δ9 elongase.

10. The method of claim **1**, wherein the disruption in the native gene encoding a peroxisome biogenesis factor protein comprises a deletion selected from the group consisting of:

a deletion in a portion of the gene encoding the C-terminal portion of the protein and a gene knockout.

11. The method of claim **1**, wherein the peroxisome biogenesis factor protein is selected from the group consisting of:

Pex1p, Pex 2p, Pex3p, Pex3Bp, Pex4p, Pex5p, Pex5Bp, Pex5Cp, Pex5/20p, Pex6p, Pex7p, Pex8p, Pex10p, Pex12p, Pex13p, Pex14p, Pex15p, Pex16p, Pex17p, Pex14/17p, Pex18p, Pex19p, Pex20p, Pex21p, Pex21Bp, Pex22p, Pex22p-like and Pex26p.

12. The method of claim **1**, wherein the peroxisome biogenesis factor protein is selected from the group consisting of:

peroxisome biogenesis factor 3 protein (Pex3p), peroxisome biogenesis factor 10 protein (Pex10p) and peroxisome biogenesis factor 16 protein (Pex16p), and wherein the disruption is a gene knockout.

13. The method of claim **1**, wherein the peroxisome biogenesis factor protein is selected from the group consisting of:

peroxisome biogenesis factor 2 protein (Pex2p), peroxisome biogenesis factor 10 protein (Pex10p) and peroxisome biogenesis factor 12 protein (Pex12p), and wherein the disruption is a deletion in a portion of the gene encoding the C-terminal portion of the C_3HC_4 zinc ring finger motif of the protein.

14. The oil fraction or the total lipid fraction in a PEX-disrupted organism having an increase in the weight percent of at least one polyunsaturated fatty acid relative to the weight percent of total fatty acids, wherein the increase was obtained by the method of claim **1**.

15. Use as food, feed or in an industrial application of the at least one polyunsaturated fatty acid of a PEX-disrupted organism having been increased in weight percent relative to the weight percent of total fatty acids by the method of claim **1**.

16. A PEX-disrupted *Yarrowia lipolytica*, wherein the disruption occurs in the native gene encoding a peroxisome biogenesis factor protein selected from the group consisting of Pex3p, Pex10p and Pex16p.

17. The *Yarrowia lipolytica* of claim **16** having ATCC designation ATCC PTA-8614 (strain Y4128).

18. A method of increasing the percent of at least one polyunsaturated fatty acid relative to the dry cell weight in an oleaginous eukaryotic organism, comprising:

a) providing an oleaginous eukaryotic organism comprising:

1) genes encoding a functional polyunsaturated fatty acid biosynthetic pathway; and

2) a disruption in a native gene encoding a peroxisome biogenesis factor protein, thereby providing a PEX-disrupted organism, and

b) growing the PEX-disrupted organism under conditions as to increase the percent of at least one polyunsaturated fatty acid relative to the dry cell weight, when compared to the percent of the at least one polyunsaturated fatty acid relative to the dry cell weight in the oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

19. The method of claim **18**, wherein the increase in the percent of the at least one polyunsaturated fatty acid relative to the dry cell weight is at least 1.3 fold, when compared to the percent of polyunsaturated fatty acids relative to the dry cell weight of an oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

20. The method of claim **19**, wherein the at least one polyunsaturated fatty acid is selected from the group consisting of:

linoleic acid, conjugated linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, docosatetraenoic acid, ω-6 docosapentaenoic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, ω-3 docosapentaenoic acid, eicosadienoic acid, eicosatrienoic acid, docosahexaenoic acid, hydroxylated or epoxy fatty acids of these, C_{18} polyunsaturated fatty acids, C_{20} polyunsaturated fatty acids, and C_{22} polyunsaturated fatty acids.

21. The method of claim **19**, wherein the total lipid content in the PEX-disrupted organism is altered, when compared with the total lipid content in an oleaginous eukaryotic organism in which no native gene encoding a peroxisome biogenesis factor protein has been disrupted.

22. The method of claim **19**, wherein the

disruption in the native gene encoding a peroxisome biogenesis factor protein comprises a deletion selected from the group consisting of:

a deletion in a portion of the gene encoding the C-terminal portion of the protein, and a gene knockout; and

wherein the peroxisome biogenesis factor protein is selected from the group consisting of:

Pex1p, Pex 2p, Pex3p, Pex3Bp, Pex4p, Pex5p, Pex5Bp, Pex5Cp, Pex5/20p, Pex6p, Pex7p, Pex8p, Pex10p, Pex12p, Pex13p, Pex14p, Pex15p, Pex16p, Pex17p, Pex14/17p, Pex18p, Pex19p, Pex20p, Pex21p, Pex21Bp, Pex22p, Pex22p-like and Pex26p.

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