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NOVEL ACYLTRANSFERASES, VARIANT THIOESTERASES, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 **[0001]** This application claims benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 62/404,667, filed October 5, 2016, and titled “NOVEL ACYLTRANSFERASES, VARIANT THIOESTERASES, AND USES THEREOF,” and U.S. Patent Application No. 15/725,222, filed October 4, 2017, and titled, “NOVEL ACYLTRANSFERASES, VARIANT THIOESTERASES,
- 10 AND USES THEREOF,” both of which are hereby incorporated herein by reference herein in their entireties.

REFERENCE TO A SEQUENCE LISTING

- 15 **[0002]** This application includes a list of sequences, as shown at the end of the detailed description.

FIELD OF THE INVENTION

- 20 **[0003]** Embodiments of the present invention relate to oils/fats, fuels, foods, and oleochemicals and their production from cultures of genetically engineered cells. Embodiments relate to nucleic acids and proteins that are involved in the fatty acid synthetic pathways; oils with a high content of triglycerides bearing fatty acyl groups upon the glycerol backbone in particular regiospecific patterns, highly stable oils, oils with high levels of oleic or mid-chain fatty acids, and products produced from such oils.

BACKGROUND OF THE INVENTION

- 25 **[0004]** Co-owned patent applications WO2008/151149, WO2010/063031, WO2010/063032, WO2011/150410, WO2011/150411, WO2012/061647, WO2012/061647, WO2012/106560, WO2013/158938, WO2014/120829, WO2014/151904, WO2015/051319, WO2016/007862, WO2016/014968, WO2016/044779, and WO2016/164495 relate to microbial oils and methods for

producing those oils in host cells, including microalgae. These publications also describe the use of such oils to make foods, oleochemicals, fuels and other products.

[0005] Certain enzymes of the fatty acyl-CoA elongation pathway function to extend the length of fatty acyl-CoA molecules. Elongase-complex enzymes extend fatty acyl-CoA molecules in 2 carbon additions, for example myristoyl-CoA to palmitoyl-CoA, stearoyl-CoA to arachidyl-CoA, or oleoyl-CoA to eicosanoyl-CoA, eicosanoyl-CoA to erucyl-CoA. In addition, elongase enzymes also extend acyl chain length in 2 carbon increments. KCS enzymes condense acyl-CoA molecules with two carbons from malonyl-CoA to form beta-ketoacyl-CoA. KCS and elongases may show specificity for condensing acyl substrates of particular carbon length, modification (such as hydroxylation), or degree of saturation. For example, the jojoba (*Simmondsia chinensis*) beta-ketoacyl-CoA synthase has been demonstrated to prefer monounsaturated and saturated C18- and C20-CoA substrates to elevate production of erucic acid in transgenic plants (Lassner et al., *Plant Cell*, 1996, Vol 8(2), pp. 281-292), whereas specific elongase enzymes of *Trypanosoma brucei* show preference for elongating short and midchain saturated CoA substrates (Lee et al., *Cell*, 2006, Vol 126(4), pp. 691-9).

[0006] The type II fatty acid biosynthetic pathway employs a series of reactions catalyzed by soluble proteins with intermediates shuttled between enzymes as thioesters of acyl carrier protein (ACP). By contrast, the type I fatty acid biosynthetic pathway uses a single, large multifunctional polypeptide.

[0007] The oleaginous, non-photosynthetic alga, *Prototheca moriformis*, stores copious amounts of triacylglyceride oil under conditions when the nutritional carbon supply is in excess, but cell division is inhibited due to limitation of other essential nutrients. Bulk biosynthesis of fatty acids with carbon chain lengths up to C18 occurs in the plastids; fatty acids are then exported to the endoplasmic reticulum where (if it occurs) elongation past C18 and incorporation into triacylglycerides (TAGs) is believed to occur. Lipids are stored in large cytoplasmic organelles called lipid bodies until environmental conditions change to favor growth, whereupon they are mobilized to provide energy and carbon molecules for anabolic metabolism.

SUMMARY OF THE INVENTION

[0008] In various aspects, the inventions disclosed herein include one or more of the following embodiments. The embodiments can be practiced alone or in combination with each other.

- 5 [0009] Embodiment 1: This embodiment of the invention provides a recombinant vector construct or a host cell comprising nucleic acids that encode an acyltransferase that optionally is operable to produce an altered fatty acid profile or an altered *sn*-2 profile in an oil produced by a host cell expressing the nucleic acids. The nucleic acids can be a nucleic acid construct or a vector construct that also includes one or
- 10 more regulatory elements. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The acyltransferase encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID
- 15 NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196. The acyl transferases of this invention is a
- 20 lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2). The acyltransferases of the invention are shown in Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least
- 25 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In another embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, or 99% identity to an acyltransferase of clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least
- 30 86.5%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 3 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%,

or 99% identity to an acyltransferase of clade 4 of Table 5. In one embodiment, the recombinant vector construct of host cell comprises nucleic acids that 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase encoded by SEQ ID NOs: 19, 20, 21, 22, 5 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125.

[0010] Embodiment 2: This embodiment of the invention provides nucleic acids that encode an acyltransferase that when expressed produces an altered fatty acid 10 profile or an altered *sn*-2 profile in an oil produced by a host cell expressing the nucleic acids. The nucleic acids can be a nucleic acid construct or a vector construct that also includes one or more regulatory elements. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The 15 acyltransferase encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 20 82, 83, 84, 85, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196. The acyl transferases of this invention is a lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2). The 25 acyltransferases of the invention are shown in Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In another embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, 30 or 99% identity to an acyltransferase of clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 86.5%, 90%, 95%, 98%, or 99% identity to an

- acyltransferase of clade 3 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 4 of Table 5. In one embodiment, the nucleic acids comprise nucleic acids that are 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase encoded by SEQ ID NOs: 19, 20, 21, 22, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125.
- 10 **[0011]** Embodiment 3: This embodiment of the invention provides codon-optimized nucleic acids that encodes an acyltransferase operable to produce an altered fatty acid profile and/or an altered *sn*-2 profile in an oil produced by a host cell expressing the nucleic acids. In one aspect, the codons are optimized for expression in the host cell, including host cells derived from plants. In another aspect, the codons are optimized for expression in *Prototheca* or *Chlorella*. In a further aspect the codons are optimized for expression in *Prototheca moriformis* or *Chlorella protothecoides*. The codon-optimized nucleic acids can be a nucleic acid construct or a vector construct that also includes one or more regulatory elements. The one or more regulatory elements are also codon-optimized for *Prototheca* or *Chlorella*. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The acyltransferase encoded by the codon-optimized nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196. When the codons are optimized for expression in a host organism, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codons used is the most preferred codon. Alternately, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codons used is the first or second most

preferred codon. The codon-optimized nucleic acids encode acyltransferases that are shown in Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In another embodiment,

5 the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, or 99% identity to an acyltransferase of clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 86.5%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 3 of Table 5. In one

10 embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 4 of Table 5. The acyltransferase encoded by the codon-optimized nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an

15 acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196. In one

20 embodiment, the codon-optimizes nucleic acids comprise nucleic acids that 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase encoded by SEQ ID NOs: 19, 20, 21, 22, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122,

25 123, 124, or 125.

[0012] Embodiment 4: In this embodiment, the invention provides host cells that are oleaginous microorganism cells or plant cells. The microorganisms of the invention are eukaryotic microorganism. In one aspect, the host cells are microalgae. In one embodiment, the microalgae are of the phylum Chlorophyta, the class

30 Trebouxiophytae, the order Chlorellales, or the family Chlorellaceae. In one embodiment, the microalgae are of the genus *Prototheca* or *Chlorella*. In one embodiment, the microalgae are of the species *Prototheca moriformis*, *Prototheca*

zopfii, *Prototheca wickerhamii* *Prototheca blaschkeae*, *Prototheca chlorelloides*, *Prototheca crieana*, *Prototheca dilamenta*, *Prototheca hydrocarborea*, *Prototheca kruegeri*, *Prototheca portoricensis*, *Prototheca salmonis*, *Prototheca segbwema*, *Prototheca stagnorum*, *Prototheca trispora* *Prototheca ulmea*, or *Prototheca viscosa*.

- 5 Preferably, the microalga is of the species *Prototheca moriformis*. In one embodiment, the microalgae are of the species *Chlorella autotrophica*, *Chlorella colonials*, *Chlorella lewinii*, *Chlorella minutissima*, *Chlorella pituitam*, *Chlorella pulchelloides*, *Chlorella pyrenoidosa*, *Chlorella rotunda*, *Chlorella singularis*, *Chlorella sorokiniana*, *Chlorella variabilis*, or *Chlorella volutis*. Preferably, the
- 10 microalga is of the species *Chlorella protothecoides* or *Auxenochlorella protothecoides*. The host cells express the nucleic acids for Embodiments relating to acyltransferases of the invention.

- [0013]** Embodiment 5: In this embodiment, the acyl transferase is lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl
- 15 glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2). In one embodiment, the acyltransferases of the invention are shown in Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least
- 20 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In another embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, or 99% identity to an acyltransferase of clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least
- 25 86.5%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 3 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 4 of Table 5. The acyltransferase have
- 30 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174,

175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.

[0014] Embodiment 6: In this embodiment, nucleic acids encoding acyltransferases increases the production of C8:0 and/or C10:0 fatty acids or alters the *sn-2* profile in the host cell. The acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In another embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, or 99% identity to an acyltransferase of clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 86.5%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 3 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 4 of Table 5. The C8:0 or the C10:0 content of the oil of the host cell is increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, or higher as compared the C8:0 and/or C10:0 content of a cell oil that does not express the recombinant nucleic acids encoding the LPAATs of the invention. The *sn-2* profile of the oil is altered by the expression of the LPAATs of the invention and/or the C8:0 and/or C10:0 fatty acid at the *sn-2* position is increased by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, or higher as compared to the C8:0 and/or C10:0 fatty acid at the *sn-2* position of the cell oil that does not express the recombinant nucleic acids encoding the LPAATs of the invention. The acyltransferase encoded by the codon-optimized nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.

[0015] Embodiment 7: This embodiment comprises nucleic acids encoding LPAATs, shown in Table 5, and disclosed herein. The LPAATs encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or 180.

[0016] Embodiment 8: In this embodiment, nucleic acids encoding GPATs of the invention have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 181, 182, 183, 184, 185, or 186.

[0017] Embodiment 9: In this embodiment, nucleic acids encoding DGATs of the invention have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 187, or 188.

[0018] Embodiment 10: In this embodiment, nucleic acids encoding LPCATs of the invention have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 189, 190, 191, or 192,

[0019] Embodiment 11: This embodiment comprises nucleic acids encoding PLA2s. The PLA2s encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 193, 194, 195, or 196.

[0020] Embodiment 12: This embodiment is a method of cultivating a host cell expressing nucleic acids that encode the one or more acyl transferases of embodiments 1-11

[0021] Embodiment 13: This embodiment is a method of producing an oil by cultivating host cells that express nucleic acids that encode the one or more acyl transferases of Embodiments 1-12 and recovering the oil.

[0022] Embodiment 14: This embodiment is an oil produced by cultivating host cells that express the one or more nucleic acids that encode the acyltransferases of

Examples 1-11, and recovering the oil from the host cell. When the host cell is a microalgae, the cell oil produced by the host cell has sterols that are different than the sterols produced by a plant cell. The cell oil has a sterol profile that is different than an oil obtained from a plant.

- 5 **[0023]** Embodiment 15: In this embodiment, a recombinant acyltransferase is provided. The recombinant acyltransferase can be produced by a host cell. The glycosylation of the recombinant acyl transferase is altered from the glycosylation pattern observed in the acyl transferase produced by the non-recombinant, wild-type cell from which the gene encoding the acyl transferase was derived. In one
- 10 embodiment, the recombinant acyltransferase the invention have acyltransferase activity and the amino acid sequence comprises at least 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In one embodiment, the recombinant acyltransferase the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, or 99% identity to an acyltransferase of
- 15 clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 86.5%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 3 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%, or
- 20 99% identity to an acyltransferase of clade 4 of Table 5. The acyltransferase encoded have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69,
- 25 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.

- [0024]** Embodiment 16: This embodiment of the invention provides a recombinant vector construct or a host cell comprising nucleic acids that encode a variant *Brassica*
- 30 fatty acyl-ACP thioesterase that optionally is operable to produce an altered fatty acid profile in an oil produced by a host cell expressing the nucleic acids. The nucleic acids can be a nucleic acid construct or a vector construct that also includes one or

more regulatory elements. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The thioesterase encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%,
 5 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 165, 166, 167, or 168 and comprise one or more of amino acid variants D124A, D209A, D127A or D212A. In one embodiment, the *Brassica Rapa*, *Brassica napus* or the *Brassica juncea* thioesterases of the invention have fatty acyl hydrolysis activity and prefer to hydrolyze long chain fatty acyl groups from the acyl carrier protein. In one
 10 embodiment, the thioesterase genes, isolated from higher plants, are altered to create variant thioesterases that have certain amino acids that have been altered from the wild type enzyme. Due to the altered amino acid(s), the substrate specificity of the thioesterase is altered. The variant BnOTE enzymes increased C18:0 content by DCW, decreased C18:1 content by DCW, and decreased C18:2 content by DCW in
 15 host cells and the oils recovered from the host cells.

[0025] Embodiment 17: This embodiment of the invention provides a recombinant vector construct or a host cell comprising nucleic acids that encode a *Garcinia mangostana* variant fatty acyl-ACP thioesterase (GmFATA) that optionally is operable to produce an altered fatty acid profile in an oil produced by a host cell
 20 expressing the nucleic acids. The nucleic acids can be a nucleic acid construct or a vector construct that also includes one or more regulatory elements. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The variant *Garcinia* thioesterase encoded by the nucleic acids have 75%, 80%,
 25 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, comprise one more of amino acid variants D variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V T156F, T156A, T156K, T156V, or V193A. In one embodiment, the *G mangostana* thioesterases of
 30 the invention have fatty acyl hydrolysis activity and prefer to hydrolyze long chain fatty acyl groups from the acyl carrier protein. In one embodiment, the thioesterase genes, isolated from higher plants, are altered to create variant thioesterases that have

certain amino acids that have been altered from the wild type enzyme. Due to the altered amino acid(s), the substrate specificity of the thioesterase is altered. The variant BnOTE enzymes increased C18:0 content by DCW, decreased C18:1 content by DCW, and decreased C18:2 content by DCW in host cells and the oils recovered from the host cells.

[0026] Embodiment 18: This embodiment of the invention provides nucleic acids that encode variant *Brassica* thioesterases or variant *Garcinia* thioesterases that when expressed produce an altered fatty acid profile in an oil produced by a host cell expressing the nucleic acids. The nucleic acids can be a nucleic acid construct or a vector construct that also includes one or more regulatory elements. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The variant *Brassica* thioesterases encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 165, 166, 167, or 168 and comprise one or more of amino acid variants D124A, D209A, D127A or D212A. The variant variant *Garcinia* thioesterases encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and comprise one or more of amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V T156F, T156A, T156K, T156V, or V193A.

[0027] Embodiment 19: This embodiment of the invention provides codon-optimized nucleic acids that encodes a variant *Brassica* thioesterase or a variant *Garcinia* thioesterase operable to produce an altered fatty acid profile in an oil produced by a host cell expressing the nucleic acids. In one aspect, the codons are optimized for expression in the host cell, including host cells derived from plants. In another aspect, the codons are optimized for expression in *Prototheca* or *Chlorella*. In a further aspect the codons are optimized for expression in *Prototheca moriformis* or *Chlorella protothecoides*. The codon-optimized nucleic acids can be a nucleic acid construct or a vector construct that also includes one or more regulatory elements. The one or more regulatory elements are also codon-optimized for *Prototheca* or

Chlorella. The one or more regulatory elements include promoters, targeting sequences, secretion signals and other elements that control or direct the expression of the encoded protein in the host cell. The variant *Brassica* thioesterases encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 165, 166, 167, or 168 and comprise one or more of amino acid variants D124A, D209A, D127A or D212A. The variant variant *Garcinia* thioestrases encoded by the nucleic acids have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 and comprise one or more of amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V T156F, T156A, T156K, T156V, or V193A. When the codons are optimized for expression in a host organism, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codons used is the most preferred codon. Alternately, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codons used is the first or second most preferred codon. The codon-optimized nucleic acids encode variant *Brassica* thioesterases and variant *Garcinia* thioestrases. In one embodiment, the variant *Brassica* thioesterases and variant *Garcinia* thioestrases of the invention have thioesterase activity.

[0028] Embodiment 20: In this embodiment, the invention provides host cells that are oleaginous microorganism cells or plant cells. The microorganisms of the invention are eukaryotic microorganism. In one aspect, the host cells are microalgae. In one embodiment, the microalgae are of the phylum Chlorophyta, the class Trebouxiophytae, the order Chlorellales, or the family Chlorellaceae. In one embodiment, the microalgae are of the genus *Prototheca* or *Chlorella*. In one embodiment, the microalgae are of the species *Prototheca moriformis*, *Prototheca zopfii*, *Prototheca wickerhamii* *Prototheca blaschkeae*, *Prototheca chlorelloides*, *Prototheca crieana*, *Prototheca dilamenta*, *Prototheca hydrocarborea*, *Prototheca kruegeri*, *Prototheca portoricensis*, *Prototheca salmonis*, *Prototheca segbwema*, *Prototheca stagnorum*, *Prototheca trispora* *Prototheca ulmea*, or *Prototheca viscosa*. Preferably, the microalga is of the species *Prototheca moriformis*. In one embodiment, the microalgae are of the species *Chlorella autotrophica*, *Chlorella*

colonials, Chlorella lewinii, Chlorella minutissima, Chlorella pituitam, Chlorella pulchelloides, Chlorella pyrenoidosa, Chlorella rotunda, Chlorella singularis, Chlorella sorokiniana, Chlorella variabilis, or Chlorella volutis. Preferably, the microalga is of the species *Chlorella protothecoides* or *Auxenochlorella*

5 *protothecoides.* The host cells express the nucleic acids for Embodiments relating to acyltransferases of the invention.

[0029] Embodiment 21: In this embodiment, the nucleic acid encoding the variant *Brassica* thioesterase encodes a variant thioesterase that has 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or
10 100% identity to SEQ ID NOs: 165, 166, 167, or 168 and comprise one or more of amino acid variants D124A, D209A, D127A or D212A.. In another aspect, the nucleic acid encoding the variant *Garcinia* thioesterase encodes a variant thioesterase that has 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%,
15 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150, and comprise one or more of amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V T156F, T156A, T156K, T156V, or V193A..

[0030] Embodiment 22: In this embodiment, nucleic acids encoding a variant *Brassica* thioesterase or a variant *Garcinia* thioesetrase that decrease the production
20 of C18:0 and/or decrease the production of C18:1 fatty acids and/or decreases the production of C18:2 fatty acids *sn-2* in the host cell.

[0031] Embodiment 23: In this embodiment, nucleic acids encoding a variant *Brassica* thioesterase of the invention have SEQ ID NOs: 165, 166, 167, or 168 and comprise one or more of amino acid variants D124A, D209A, D127A or D212A.

25 [0032] Embodiment 24: In this embodiment, nucleic acids encoding a variant *Garcinia* thioesetrase of the invention have 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 and comprise one or more of amino acid variants L91F, L91K, L91S, G96A, G96T,
30 G96V, G108A, G108V, S111A, S111V T156F, T156A, T156K, T156V, or V193A.

[0033] Embodiment 25: This embodiment is a method of cultivating a host cell expressing nucleic acids that encode the one or more acyl transferases of embodiments 16-24.

5 [0034] Embodiment 26: This embodiment is a method of producing an oil by cultivating host cells that express nucleic acids that encode the one or more variant thioesterases of Embodiments 16-25 and recovering the oil.

[0035] Embodiment 27: This embodiment is an oil produced by cultivating host cells that express the one or more nucleic acids that encode the variant transferases of Examples 16-24, and recovering the oil from the host cell. When the host cell is a
10 microalgae, the cell oil produced by the host cell has sterols that are different than the sterols produced by a plant cell. The cell oil has a sterol profile that is different than an oil obtained from a plant.

[0036] Embodiment 28: In this embodiment, a recombinant variant thioesterase is provided. The recombinant variant thioesterase is produce by a host cell. The
15 glycosylation of the recombinant variant thioesterase is altered from the glycosylation pattern observed in the variant thioesterase produced by the non-recombinant, wild-type cell from which the gene encoding the variant thioesterase was derived.

[0037] By way of example and not intended to be the only combination, the acyltransferase and/or the variant acyl-ACP thioesterrases of the invention can be
20 expressed in a cell in which an endogenous desaturase, KAS, and/or fatty acyl-ACP thioesterase has been ablated or downregulated as demonstrated in the Examples. The co-expression of an acyltransferase and/or a variant acyl-ACP thioesterase concomitantly with an invertase is an embodiment of the invention, as was demonstrated in the disclosed Examples. Additionally, the expression of an
25 acyltansferase and/or a variant acyl-ACP thioesterase with concomitant expression of a invertase and ablation or downregulation of a desaturase, KAS and/or fatty acyl-ACP thioesterase is an embodiment of the invention, as demonstrated in the disclosed Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

30 [0038] **FIG 1. TAG profiles of S7815 versus the S6573 parent.** TAGs in brackets co-elute with the peak of the main TAG, but are present in trace amounts, and do not

contribute significantly to the area. M = myristate (C14:0), P = palmitate (C16:0), Po = palmitoleate (C16:1), Ma = margaric (C17:0), S = stearate (C18:0), O = oleate (C18:1), L = linoleate (C18:2), Ln = linolenate (C18:3 α), A = arachidate (C20:0), B = behenate (C22:0), Lg = lignocerate (C24:0), Hx = hexacosanoate (C26:0). Sat-Sat-Sat = trisaturates. See Example 5.

[0039] FIG 2. TAG profiles of lipids from fermentations of S7815 versus S6573. TAGs in brackets co-elute with the peak of the main TAG, but are present in trace amounts, and do not contribute significantly to the area. M = myristate (C14:0), P = palmitate (C16:0), S = stearate (C18:0), O = oleate (C18:1), L = linoleate (C18:2), Ln = linolenate (C18:3 α), A = arachidate (C20:0), B = behenate (C22:0), Lg = lignocerate (C24:0), Hx = hexacosanoate (C26:0). Sat-Sat-Sat = trisaturates. See Example 5.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

[0040] An “allele” refers to a copy of a gene where an organism has multiple similar or identical gene copies, even if on the same chromosome. An allele may encode the same or similar protein.

[0041] An “oil,” “cell oil” or “cell fat” shall mean a predominantly triglyceride oil obtained from an organism, where the oil has not undergone blending with another natural or synthetic oil, or fractionation so as to substantially alter the fatty acid profile of the triglyceride. In connection with an oil comprising triglycerides of a particular regiospecificity, the cell oil or cell fat has not been subjected to interesterification or other synthetic process to obtain that regiospecific triglyceride profile, rather the regiospecificity is produced naturally, by a cell or population of cells. For a cell oil produced by a cell, the sterol profile of oil is generally determined by the sterols produced by the cell, not by artificial reconstitution of the oil by adding sterols in order to mimic the cell oil. In connection with a cell oil or cell fat, and as used generally throughout the present disclosure, the terms oil, and fat are used interchangeably, except where otherwise noted. Thus, an “oil” or a “fat” can be liquid, solid, or partially solid at room temperature, depending on the makeup of the substance and other conditions. Here, the term “fractionation” means removing

material from the oil in a way that changes its fatty acid profile relative to the profile produced by the organism, however accomplished. The terms “oil,” “cell oil” and “cell fat” encompass such oils obtained from an organism, where the oil has undergone minimal processing, including refining, bleaching, deodorized, and/or degumming, which does not substantially change its triglyceride profile. A cell oil can also be a “noninteresterified cell oil”, which means that the cell oil has not undergone a process in which fatty acids have been redistributed in their acyl linkages to glycerol and remain essentially in the same configuration as when recovered from the organism.

10 **[0042]** As used herein, an oil is said to be “enriched” in one or more particular fatty acids if there is at least a 10% increase in the mass of that fatty acid in the oil relative to the non-enriched oil. For example, in the case of a cell expressing a heterologous FatB gene described herein, the oil produced by the cell is said to be enriched in, e.g., C8 and C16 fatty acids if the mass of these fatty acids in the oil is at least 10% greater than in oil produced by a cell of the same type that does not express the heterologous FatB gene (e.g., wild type oil).

15 **[0043]** “Exogenous gene” shall mean a nucleic acid that codes for the expression of an RNA and/or protein that has been introduced into a cell (*e.g.* by transformation/transfection), and is also referred to as a “transgene”. A cell comprising an exogenous gene may be referred to as a recombinant cell, into which additional exogenous gene(s) may be introduced. The exogenous gene may be from a different species (and so heterologous), or from the same species (and so homologous), relative to the cell being transformed. Thus, an exogenous gene can include a homologous gene that occupies a different location in the genome of the cell or is under different control, relative to the endogenous copy of the gene. An exogenous gene may be present in more than one copy in the cell. An exogenous gene may be maintained in a cell as an insertion into the genome (nuclear or plastid) or as an episomal molecule.

25 **[0044]** “FADc”, also referred to as “FAD2” or “FAD” is a gene encoding a delta-12 fatty acid desaturase. “SAD” is a gene encoding a stearoyl ACP desaturase, a delta-9 fatty acid desaturase. The desaturases desaturates a fatty acyl chain to create a

double bond. SAD converts stearic acid, C18:0 to oleic acid, C18:1 and FAD converts oleic acid, C18:1 to linoleic acid, C18:2.

[0045] “Fatty acids” shall mean free fatty acids, fatty acid salts, or fatty acyl moieties in a glycerolipid. It will be understood that fatty acyl groups of glycerolipids can be described in terms of the carboxylic acid or anion of a carboxylic acid that is produced when the triglyceride is hydrolyzed or saponified.

[0046] “Fixed carbon source” is a molecule(s) containing carbon, typically an organic molecule that is present at ambient temperature and pressure in solid or liquid form in a culture media that can be utilized by a microorganism cultured therein. Accordingly, carbon dioxide is not a fixed carbon source. Typical fixed carbon source include sucrose, glucose, fructose and other well-known monosaccharides, disaccharides and polysaccharides.

[0047] “In operable linkage” is a functional linkage between two nucleic acid sequences, such a control sequence (typically a promoter) and the linked sequence (typically a sequence that encodes a protein, also called a coding sequence). A promoter is in operable linkage with an exogenous gene if it can mediate transcription of the gene.

[0048] “Microalgae” are eukaryotic microbial organisms that contain a chloroplast or other plastid, and optionally that is capable of performing photosynthesis, or a prokaryotic microbial organism capable of performing photosynthesis. Microalgae include obligate photoautotrophs, which cannot metabolize a fixed carbon source as energy, as well as heterotrophs, which can live solely off of a fixed carbon source. Microalgae also include mixotrophic organisms that can perform photosynthesis and metabolize one or more fixed carbon source. Microalgae include unicellular organisms that separate from sister cells shortly after cell division, such as *Chlamydomonas*, as well as microbes such as, for example, *Volvox*, which is a simple multicellular photosynthetic microbe of two distinct cell types. Microalgae include cells such as *Chlorella*, *Dunaliella*, and *Prototheca*. Microalgae also include other microbial photosynthetic organisms that exhibit cell-cell adhesion, such as *Agmenellum*, *Anabaena*, and *Pyrobotrys*. Microalgae also include obligate heterotrophic microorganisms that have lost the ability to perform photosynthesis, such as certain dinoflagellate algae species and species of the genus *Prototheca*.

[0049] As used with respect to nucleic acids, the term “isolated” refers to a nucleic acid that is free of at least one other component that is typically present with the naturally occurring nucleic acid. Thus, a naturally occurring nucleic acid is isolated if it has been purified away from at least one other component that occurs naturally with the nucleic acid.

[0050] In connection with fatty acid length, “mid-chain” shall mean C8 to C16 fatty acids.

[0051] In connection with a recombinant cell, the term “knockdown” refers to a gene that has been partially suppressed (e.g., by about 1-95%) in terms of the production or activity of a protein encoded by the gene. Inhibitory RNA technology to down-regulate or knockdown expression of a gene are well known. These techniques include dsRNA, hairpin RNA, antisense RNA, interfering RNA (RNAi) and others.

[0052] Also, in connection with a recombinant cell, the term “knockout” refers to a gene that has been completely or nearly completely (e.g., >95%) suppressed in terms of the production or activity of a protein encoded by the gene. Knockouts can be prepared by ablating the gene by homologous recombination of a nucleic acid sequence into a coding sequence, gene deletion, mutation or other method. When homologous recombination is performed, the nucleic acid that is inserted (“knocked-in”) can be a sequence that encodes an exogenous gene of interest or a sequence that does not encode for a gene of interest. The ablation by homologous recombination can be performed in one, two or more alleles of the gene of interest.

[0053] An “oleaginous” cell is a cell capable of producing at least 20% lipid by dry cell weight, naturally or through recombinant or classical strain improvement. An “oleaginous microbe” or “oleaginous microorganism” is a microbe, including a microalga that is oleaginous (especially eukaryotic microalgae that store lipid). An oleaginous cell also encompasses a cell that has had some or all of its lipid or other content removed, and both live and dead cells.

[0054] An “ordered oil” or “ordered fat” is one that forms crystals that are primarily of a given polymorphic structure. For example, an ordered oil or ordered fat can have

crystals that are greater than 50%, 60%, 70%, 80%, or 90% of the β or β' polymorphic form.

[0055] In connection with a cell oil, a “profile” is the distribution of particular species or triglycerides or fatty acyl groups within the oil. A “fatty acid profile” is the distribution of fatty acyl groups in the triglycerides of the oil without reference to attachment to a glycerol backbone. Fatty acid profiles are typically determined by conversion to a fatty acid methyl ester (FAME), followed by gas chromatography (GC) analysis with flame ionization detection (FID), as in Example 1. The fatty acid profile can be expressed as one or more percent of a fatty acid in the total fatty acid signal determined from the area under the curve for that fatty acid. FAME-GC-FID measurement approximate weight percentages of the fatty acids. A “*sn*-2 profile” is the distribution of fatty acids found at the *sn*-2 position of the triacylglycerides in the oil. A “regiospecific profile” is the distribution of triglycerides with reference to the positioning of acyl group attachment to the glycerol backbone without reference to stereospecificity. In other words, a regiospecific profile describes acyl group attachment at *sn*-1/3 vs. *sn*-2. Thus, in a regiospecific profile, POS (palmitate-oleate-stearate) and SOP (stearate-oleate-palmitate) are treated identically. A “stereospecific profile” describes the attachment of acyl groups at *sn*-1, *sn*-2 and *sn*-3. Unless otherwise indicated, triglycerides such as SOP and POS are to be considered equivalent. A “TAG profile” is the distribution of fatty acids found in the triglycerides with reference to connection to the glycerol backbone, but without reference to the regiospecific nature of the connections. Thus, in a TAG profile, the percent of SSO in the oil is the sum of SSO and SOS, while in a regiospecific profile, the percent of SSO is calculated without inclusion of SOS species in the oil. In contrast to the weight percentages of the FAME-GC-FID analysis, triglyceride percentages are typically given as mole percentages; that is the percent of a given TAG molecule in a TAG mixture.

[0056] The term “percent sequence identity,” in the context of two or more amino acid or nucleic acid sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. For sequence

comparison to determine percent nucleotide or amino acid identity, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence
5 algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Optimal alignment of sequences for comparison can be conducted using the NCBI BLAST software (ncbi.nlm.nih.gov/BLAST/) set to default parameters. For example, to compare two
10 nucleic acid sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (Apr. 21, 2000) set at the following default parameters: Matrix: BLOSUM62; Reward for match: 1; Penalty for mismatch: -2; Open Gap: 5 and Extension Gap: 2 penalties; Gap x drop-off: 50; Expect: 10; Word Size: 11; Filter: on. For a pairwise comparison of two amino acid sequences, one may use the "BLAST 2
15 Sequences" tool Version 2.0.12 (Apr. 21, 2000) with blastp set, for example, at the following default parameters: Matrix: BLOSUM62; Open Gap: 11 and Extension Gap: 1 penalties; Gap x drop-off 50; Expect: 10; Word Size: 3; Filter: on.

[0057] "Recombinant" is a cell, nucleic acid, protein or vector that has been modified due to the introduction of an exogenous nucleic acid or the alteration of a
20 native nucleic acid. Thus, e.g., recombinant cells can express genes that are not found within the native (non-recombinant) form of the cell or express native genes differently than those genes are expressed by a non-recombinant cell. Recombinant cells can, without limitation, include recombinant nucleic acids that encode for a gene product or for suppression elements such as mutations, knockouts, antisense,
25 interfering RNA (RNAi), hairpin RNA or dsRNA that reduce the levels of active gene product in a cell. A "recombinant nucleic acid" is a nucleic acid originally formed in vitro, in general, by the manipulation of nucleic acid, e.g., using polymerases, ligases, exonucleases, and endonucleases, using chemical synthesis, or otherwise is in a form not normally found in nature. Recombinant nucleic acids may be produced, for
30 example, to place two or more nucleic acids in operable linkage. Thus, an isolated nucleic acid or an expression vector formed in vitro by ligating DNA molecules that are not normally joined in nature, are both considered recombinant for the purposes of

this invention. Once a recombinant nucleic acid is made and introduced into a host cell or organism, it may replicate using the in vivo cellular machinery of the host cell; however, such nucleic acids, once produced recombinantly, although subsequently replicated intracellularly, are still considered recombinant for purposes of this

5 invention. Similarly, a “recombinant protein” is a protein made using recombinant techniques, *i.e.*, through the expression of a recombinant nucleic acid. A recombinant protein will have a different pattern of glycosylation than the protein isolated from the wild-type organism.

[0058] The genes can be used in a variety of genetic constructs including plasmids
10 or other vectors for expression or recombination in a host cell. The genes can be codon optimized for expression in a target host cell. The proteins produced by the genes can be used in vivo or in purified form.

[0059] For example, the gene can be prepared in an expression vector comprising an operably linked promoter and 5'UTR. Where a plastidic cell is used as the host, a
15 suitably active plastid targeting peptide can be fused to the FATB gene, as in the examples below. Generally, for the newly identified FATB genes, there are roughly 50 amino acids at the N-terminal that constitute a plastid transit peptide, which are responsible for transporting the enzyme to the chloroplast. In the examples below, this transit peptide is replaced with a 38 amino acid sequence that is effective in the
20 *Prototheca moriformis* host cell for transporting the enzyme to the plastids of those cells. Thus, the invention contemplates deletions and fusion proteins in order to optimize enzyme activity in a given host cell. For example, a transit peptide from the host or related species may be used instead of that of the newly discovered plant genes described here.

25 [0060] A selectable marker gene may be included in the vector to assist in isolating a transformed cell. Examples of selectable markers useful in microlagae include sucrose invertase antibiotic resistance genes and other genes useful as selectable markers. The *S.carlbergensis* MEL1 gene (conferring the ability to grow on melibiose), *A. thaliana* THIC gene (conferring the ability to grow in media free of
30 thiamine, *Saccharomyces* sucrose invertase (conferring the ability to grow on sucrose) are disclosed in the Examples. Other known selectable markers are useful and within the ambit of a skilled artisan.

[0061] The terms “triglyceride”, “triacylglyceride” and “TAG” are used interchangeably as is known in the art.

II. EMBODIMENTS OF THE INVENTION

[0062] Illustrative embodiments of the present invention feature oleaginous cells that produce altered fatty acid profiles and/or altered regiospecific distribution of fatty acids in glycerolipids, and products produced from the cells. Examples of oleaginous cells include microbial cells having a type II fatty acid biosynthetic pathway, including plastidic oleaginous cells such as those of oleaginous algae and, where applicable, oil producing cells of higher plants including but not limited to commercial oilseed crops such as soy, corn, rapeseed/canola, cotton, flax, sunflower, safflower and peanut. Other specific examples of cells include heterotrophic or obligate heterotrophic microalgae of the phylum Chlorophyta, the class Trebouxiophytae, the order Chlorellales, or the family Chlorellaceae. Examples of oleaginous microalgae and methods of cultivation are also provided in co-owned applications WO2008/151149, WO2010/063031, WO2010/063032, WO2011/150410, WO2011/150411, WO2012/061647, WO2012/061647, WO2012/106560, and WO2013/158938, WO2014/120829, WO2014/151904, WO2015/051319, WO2016/007862, WO2016/014968, WO2016/044779, WO2016/164495, all of which are incorporated by reference, including species of *Chlorella* and *Prototheca*, a genus comprising obligate heterotrophs. The oleaginous cells can be, for example, capable of producing 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, or about 90% oil by cell weight, $\pm 5\%$. Optionally, the oils produced can be low in highly unsaturated fatty acids such as DHA or EPA fatty acids. For example, the oils can comprise less than 5%, 2 %, or 1% DHA and/or EPA. The above-mentioned publications also disclose methods for cultivating such cells and extracting oil, especially from microalgal cells; such methods are applicable to the cells disclosed herein and incorporated by reference for these teachings. When microalgal cells are used they can be cultivated autotrophically (unless an obligate heterotroph) or in the dark using a sugar (e.g., glucose, fructose and/or sucrose) In any of the embodiments described herein, the cells can be heterotrophic cells comprising an exogenous invertase gene so as to allow the cells to produce oil from a sucrose feedstock. Alternately, or in addition, the cells can metabolize xylose from cellulosic feedstocks. For example, the cells can be

genetically engineered to express one or more xylose metabolism genes such as those encoding an active xylose transporter, a xylulose-5-phosphate transporter, a xylose isomerase, a xylulokinase, a xylitol dehydrogenase and a xylose reductase. See WO2012/154626, “GENETICALLY ENGINEERED MICROORGANISMS THAT
5 METABOLIZE XYLOSE”, published Nov 15, 2012, including disclosure of genetically engineered *Prototheca* strains that utilize xylose.

[0063] The host cells expressing the acyltransferases or the variant *B. napus* thioesterases or the variant *G. mangostana* thioesterase may, optionally, be cultivated in a bioreactor/fermenter. For example, heterotrophic oleaginous microalgal cells can
10 be cultivated on a sugar-containing nutrient broth. Optionally, cultivation can proceed in two stages: a seed stage and a lipid-production stage. In the seed stage, the number of cells is increased from a starter culture. Thus, the seed stage(s) typically includes a nutrient rich, nitrogen replete, media designed to encourage rapid cell division. After the seed stage(s), the cells may be fed sugar under nutrient-limiting
15 (e.g. nitrogen sparse) conditions so that the sugar will be converted into triglycerides. As used herein, “standard lipid production conditions” are disclosed here. In one embodiment, the culture conditions are nitrogen limiting. Sugar and other nutrients can be added during the fermentation but no additional nitrogen is added. The cells will consume all or nearly all of the nitrogen present, but no additional nitrogen is
20 provided. For example, the rate of cell division in the lipid-production stage can be decreased by 50%, 80%, or more relative to the seed stage. Additionally, variation in the media between the seed stage and the lipid-production stage can induce the recombinant cell to express different lipid-synthesis genes and thereby alter the triglycerides being produced. For example, as discussed below, nitrogen and/or pH
25 sensitive promoters can be placed in front of endogenous or exogenous genes. This is especially useful when an oil is to be produced in the lipid-production phase that does not support optimal growth of the cells in the seed stage.

[0064] The oleaginous cells express one or more exogenous genes encoding fatty acid biosynthesis enzymes. As a result, some embodiments feature cell oils that were
30 not obtainable from a non-plant or non-seed oil, or not obtainable at all.

[0065] The oleaginous cells, including microalgal cells, can be improved via classical strain improvement techniques such as UV and/or chemical mutagenesis

followed by screening or selection under environmental conditions, including selection on a chemical or biochemical toxin. For example the cells can be selected on a fatty acid synthesis inhibitor, a sugar metabolism inhibitor, or an herbicide. As a result of the selection, strains can be obtained with increased yield on sugar, increased oil production (e.g., as a percent of cell volume, dry weight, or liter of cell culture), or improved fatty acid or TAG profile. Co-owned application PCT/US2016/025023 filed on 31 March 2016, herein incorporated by reference, describes methods for classically mutagenizing oleaginous cells.

[0066] The cells can be selected on one or more of 1,2-Cyclohexanedione; 19-Norethindone acetate; 2,2-dichloropropionic acid; 2,4,5-trichlorophenoxyacetic acid; 2,4,5-trichlorophenoxyacetic acid, methyl ester; 2,4-dichlorophenoxyacetic acid; 2,4-dichlorophenoxyacetic acid, butyl ester; 2,4-dichlorophenoxyacetic acid, isooctyl ester; 2,4-dichlorophenoxyacetic acid, methyl ester; 2,4-dichlorophenoxybutyric acid; 2,4-dichlorophenoxybutyric acid, methyl ester; 2,6-dichlorobenzonitrile; 2-deoxyglucose; 5-Tetradecyloxy-w-furoic acid; A-922500; acetochlor; alachlor; ametryn; amphotericin; atrazine; benfluralin; bensulide; bentazon; bromacil; bromoxynil; Cafenstrole; carbonyl cyanide m-chlorophenyl hydrazone (CCCP); carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP); cerulenin; chlorpropham; chlorsulfuron; clofibric acid; clopyralid; colchicine; cycloate; cyclohexamide; C75; DACTHAL (dimethyl tetrachloroterephthalate); dicamba; dichloroprop ((R)-2-(2,4-dichlorophenoxy)propanoic acid); Diflufenican; dihydrojasmonic acid, methyl ester; diquat; diuron; dimethylsulfoxide; Epigallocatechin gallate (EGCG); endothall; ethalfluralin; ethanol; ethofumesate; Fenoxaprop-p-ethyl; Fluazifop-p-Butyl; fluometuron; fomesafen; foramsulfuron; gibberellic acid; glufosinate ammonium; glyphosate; haloxyfop; hexazinone; imazaquin; isoxaben; Lipase inhibitor THL ((-)-Tetrahydrolipstatin); malonic acid; MCPA (2-methyl-4-chlorophenoxyacetic acid); MCPB (4-(4-chloro-o-tolyloxy)butyric acid); mesotrione; methyl dihydrojasmonate; metolachlor; metribuzin; Mildronate; molinate; naptalam; norharman; orlistat; oxadiazon; oxyfluorfen; paraquat; pendimethalin; pentachlorophenol; PF-04620110; phenethyl alcohol; phenmedipham; picloram; Platencin; Platensimycin; prometon; prometryn; pronamide; propachlor; propanil; propazine; pyrazon; Quizalofop-p-ethyl; s-ethyl

dipropylthiocarbamate (EPTC); s,s,s-tributylphosphorotrithioate; salicylhydroxamic acid; sesamol; siduron; sodium methane arsenate; simazine; T-863 (DGAT inhibitor); tebuthiuron; terbacil; thiobencarb; tralkoxydim; triallate; triclopyr; triclosan; trifluralin; and vulpinic acid and others.

- 5 **[0067]** The oleaginous cells produce a storage oil, which is primarily triacylglyceride and may be stored in storage bodies of the cell. A raw oil may be obtained from the cells by disrupting the cells and isolating the oil. The raw oil may comprise sterols produced by the cells. Patent applications WO2008/151149, WO2010/063031, WO2010/063032, WO2011/150410, WO2011/150411,
- 10 WO2012/061647, WO2012/061647, WO2012/106560, WO2013/158938, WO2014/120829, WO2014/151904, WO2015/051319, WO2016/007862, WO2016/014968, WO2016/044779, and WO2016/164495 disclose heterotrophic cultivation and oil isolation techniques for oleaginous microalgae. For example, oil may be obtained by providing or cultivating, drying and pressing the cells. The oils
- 15 produced may be refined, bleached and deodorized (RBD) as known in the art or as described in WO2010/120939. The raw or RBD oils may be used in a variety of food, chemical, and industrial products or processes. Even after such processing, the oil may retain a sterol profile characteristic of the source. Sterol profiles of microalga and the microalgal cell oils are disclosed below. After recovery of the oil, a valuable
- 20 residual biomass remains. Uses for the residual biomass include the production of paper, plastics, absorbents, adsorbents, drilling fluids, as animal feed, for human nutrition, or for fertilizer.

- [0068]** In an embodiment of the invention nucleic acids that encode novel acyl transferases are provided. The novel acyltransferases are useful in altering the fatty
- 25 acid profile and/or altering the regiospecific profile of an oil produced by a host cell. The nucleic acids of the invention may contain control sequences upstream and downstream in operable linkage with the gene of interest. These control sequences include promoters, targeting sequences, untranslated sequences and other control elements. Nucleic acids of the invention encode acyltransferases that function in type
- 30 II fatty acid synthesis. The acyltransferase genes are isolated from higher plants and can be expressed in a wide variety of host cells. The acyltransferases include lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase

(GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2). and other lipid biosynthetic pathway genes as discussed herein. The acyltransferases of the invention are shown in Table 5. In one embodiment, the acyltransferases of the invention have

5 acyltransferase activity and the amino acid sequence comprises at least 96.3%, 98%, or 99% identity to an acyltransferase of clade 1 of Table 5. In another embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 93.9%, 98%, or 99% identity to an acyltransferase of clade 2 of Table 5. In one embodiment, the acyltransferases of the invention have

10 acyltransferase activity and the amino acid sequence comprises at least 86.5%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 3 of Table 5. In one embodiment, the acyltransferases of the invention have acyltransferase activity and the amino acid sequence comprises at least 78.5%, 80%, 85%, 90%, 95%, 98%, or 99% identity to an acyltransferase of clade 4 of Table 5. The acyltransferases when

15 expressed increase the SOS, POP, POS, SLS, PLO, and/or PLO content DCW in host cells and the oils recovered from the host cells. The acyltransferases when expressed in host cells decreases the sat-sat-sat content of the oil by DCW. The acyltransferases when expressed in host cells increases the sat-unsat-sat/ sat-sat-sat ratio of the oil by DCW.

20 **[0069]** In an embodiment of the invention nucleic acids that encode variant *Brassica napus* thioesterases (FATA) are provided. The novel thioesterases are useful in altering the fatty acid profile of an oil produced by a host cell. The variant *Brassica napus* thioesterases prefer to hydrolyze long chain fatty acyl groups from the acyl carrier protein. The nucleic acids of the invention may contain control sequences

25 upstream and downstream in operable linkage with the gene of interest. These control sequences include promoters, targeting sequences, untranslated sequences and other control elements. Nucleic acids of the invention encode thioesterases that function in type II fatty acid synthesis. The thioesterase genes, isolated from higher plants, are altered to create variant thioesterases that have certain amino acids that have been

30 altered from the wild type enzyme. Due to the altered amino acid(s), the substrate specificity of the thioesterase is altered. The variant thioesterases can be expressed in a wide variety of host cells. The nucleic acids encode the variant thioesterases having

amino acid sequences that are 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to SEQ ID NOs: ##-## and comprise one or more of amino acid variants D124A, D209A, D127A or D212A. The variant *Bn*OTE enzymes increased C18:0 content by DCW, decreased C18:1 content by DCW, and decreased C18:2 content by DCW in host cells and the oils recovered from the host cells.

[0070] In an embodiment of the invention nucleic acids that encode variant *Garcinia mangostana* thioesterases (FATA) are provided. The novel thioesterases are useful in altering the fatty acid profile of an oil produced by a host cell. The variant *Garcinia mangostana* thioesterases prefer to hydrolyze long chain fatty acyl groups from the acyl carrier protein. The nucleic acids of the invention may contain control sequences upstream and downstream in operable linkage with the gene of interest. These control sequences include promoters, targeting sequences, untranslated sequences and other control elements. Nucleic acids of the invention encode thioesterases that function in type II fatty acid synthesis. The thioesterase genes, isolated from higher plants, are altered to create variant thioesterases that have certain amino acids that have been altered from the wild type enzyme. Due to the altered amino acid(s), the substrate specificity of the thioesterase is altered. The variant thioesterases can be expressed in a wide variety of host cells. The nucleic acids encode the variant thioesterases having amino acid sequences that are 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to SEQ ID NOs: ##-## and comprise one or more of amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V, T156F, T156A, T156K, T156V, or V193A. The variant *Gm*FATA enzymes increased C18:0 content by DCW, decreased C18:1 content by DCW, and decreased C18:2 content by DCW in host cells and the oils recovered from the host cells.

[0071] The nucleic acids of the invention can be codon optimized for expression in a target host cell (e.g., using the codon usage tables of Tables 1a, 1b, 2a, and 2b. For example, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codons used can be the most preferred codon according to Tables 1a, 1b, 2a, and 2b. Alternately, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codons used can be the first or second most preferred codon according to Tables 1a,

1b, 2a, and 2b. Preferred codons for *Prototheca* strains and for *Chlorella protothecoides* are shown below in Tables 1a and 1b, respectively.

[0072] Table 1a : Preferred codon usage in *Prototheca* strains.

5	Ala	GCG	345 (0.36)	Asn	AAT	8 (0.04)
		GCA	66 (0.07)		AAC	201 (0.96)
		GCT	101 (0.11)			
		GCC	442 (0.46)	Pro	CCG	161 (0.29)
10					CCA	49 (0.09)
	Cys	TGT	12 (0.10)		CCT	71 (0.13)
		TGC	105 (0.90)		CCC	267 (0.49)
15	Asp	GAT	43 (0.12)	Gln	CAG	226 (0.82)
		GAC	316 (0.88)		CAA	48 (0.18)
	Glu	GAG	377 (0.96)	Arg	AGG	33 (0.06)
20		GAA	14 (0.04)		AGA	14 (0.02)
					CGG	102 (0.18)
	Phe	TTT	89 (0.29)		CGA	49 (0.08)
		TTC	216 (0.71)		CGT	51 (0.09)
25					CGC	331 (0.57)
	Gly	GGG	92 (0.12)			
		GGA	56 (0.07)	Ser	AGT	16 (0.03)
		GGT	76 (0.10)		AGC	123 (0.22)
		GGC	559 (0.71)		TCG	152 (0.28)
					TCA	31 (0.06)
	His	CAT	42 (0.21)		TCT	55 (0.10)
		CAC	154 (0.79)		TCC	173 (0.31)
	Ile	ATA	4 (0.01)	Thr	ACG	184 (0.38)

		ATT	30 (0.08)		ACA	24 (0.05)
		ATC	338 (0.91)		ACT	21 (0.05)
					ACC	249 (0.52)
	Lys	AAG	284 (0.98)			
5		AAA	7 (0.02)	Val	GTG	308 (0.50)
					GTA	9 (0.01)
	Leu	TTG	26 (0.04)		GTT	35 (0.06)
		TTA	3 (0.00)		GTC	262 (0.43)
		CTG	447 (0.61)			
10		CTA	20 (0.03)	Trp	TGG	107 (1.00)
		CTT	45 (0.06)			
		CTC	190 (0.26)	Tyr	TAT	10 (0.05)
					TAC	180 (0.95)
	Met	ATG	191 (1.00)			
15				Stop	TGA/TAG/TAA	

[0073] Table 1b: Preferred codon usage in *Chlorella protothecoides*.

	TTC (Phe)	TAC (Tyr)	TGC (Cys)	TGA (Stop)
	TGG (Trp)	CCC (Pro)	CAC (His)	CGC (Arg)
20	CTG (Leu)	CAG (Gln)	ATC (Ile)	ACC (Thr)
	GAC (Asp)	TCC (Ser)	ATG (Met)	AAG (Lys)
	GCC (Ala)	AAC (Asn)	GGC (Gly)	GTG (Val)
	GAG (Glu)			

25 **[0074] Table 2a:** Codon usage for *Cuphea wrightii*

UUU F 0.48 19.5 (52) UCU S 0.21 19.5 (52) UAU Y 0.45 6.4 (17) UGU C 0.41 10.5 (28)

UUC F 0.52 21.3 (57) UCC S 0.26 23.6 (63) UAC Y 0.55 7.9 (21) UGC C 0.59 15.0 (40)

	UUA L 0.07 5.2 (14) UCA S 0.18 16.8 (45) UAA * 0.33 0.7 (2) UGA * 0.33 0.7 (2)
	UUG L 0.19 14.6 (39) UCG S 0.11 9.7 (26) UAG * 0.33 0.7 (2) UGG W 1.00 15.4 (41)
	CUU L 0.27 21.0 (56) CCU P 0.48 21.7 (58) CAU H 0.60 11.2 (30) CGU R 0.09 5.6 (15)
5	CUC L 0.22 17.2 (46) CCC P 0.16 7.1 (19) CAC H 0.40 7.5 (20) CGC R 0.13 7.9 (21)
	CUA L 0.13 10.1 (27) CCA P 0.21 9.7 (26) CAA Q 0.31 8.6 (23) CGA R 0.11 6.7 (18)
	CUG L 0.12 9.7 (26) CCG P 0.16 7.1 (19) CAG Q 0.69 19.5 (52) CGG R 0.16 9.4 (25)
	AAU I 0.44 22.8 (61) ACU T 0.33 16.8 (45) AAU N 0.66 31.4 (84) AGU S 0.18 16.1 (43)
10	AUC I 0.29 15.4 (41) ACC T 0.27 13.9 (37) AAC N 0.34 16.5 (44) AGC S 0.07 6.0 (16)
	AUA I 0.27 13.9 (37) ACA T 0.26 13.5 (36) AAA K 0.42 21.0 (56) AGA R 0.24 14.2 (38)
	AUG M 1.00 28.1 (75) ACG T 0.14 7.1 (19) AAG K 0.58 29.2 (78) AGG R 0.27 16.1 (43)
	GUU V 0.28 19.8 (53) GCU A 0.35 31.4 (84) GAU D 0.63 35.9 (96) GGU G 0.29 26.6 (
15	71)
	GUC V 0.21 15.0 (40) GCC A 0.20 18.0 (48) GAC D 0.37 21.0 (56) GGC G 0.20 18.0 (48)
	GUA V 0.14 10.1 (27) GCA A 0.33 29.6 (79) GAA E 0.41 18.3 (49) GGA G 0.35 31.4 (
	84)
	GUG V 0.36 25.1 (67) GCG A 0.11 9.7 (26) GAG E 0.59 26.2 (70) GGG G 0.16 14.2 (38)
20	

[0075] Table 2b: Codon usage for Arabidopsis

	UUU F 0.51 21.8 (678320) UCU S 0.28 25.2 (782818) UAU Y 0.52 14.6 (455089) UGU C 0.60 10.5 (327640)
	UUC F 0.49 20.7 (642407) UCC S 0.13 11.2 (348173) UAC Y 0.48 13.7 (427132) UGC C 0.40 7.2 (222769)
25	UUA L 0.14 12.7 (394867) UCA S 0.20 18.3 (568570) UAA * 0.36 0.9 (29405) UGA * 0.44 1.2 (36260)

- UUG L 0.22 20.9 (649150) UCG S 0.10 9.3 (290158) UAG * 0.20 0.5 (16417) UGG W 1.00 12.5 (388049)
- CUU L 0.26 24.1 (750114) CCU P 0.38 18.7 (580962) CAU H 0.61 13.8 (428694) CGU R 0.17 9.0 (280392)
- 5 CUC L 0.17 16.1 (500524) CCC P 0.11 5.3 (165252) CAC H 0.39 8.7 (271155) CGC R 0.07 3.8 (117543)
- CUA L 0.11 9.9 (307000) CCA P 0.33 16.1 (502101) CAA Q 0.56 19.4 (604800) CGA R 0.12 6.3 (195736)
- 10 CUG L 0.11 9.8 (305822) CCG P 0.18 8.6 (268115) CAG Q 0.44 15.2 (473809) CGG R 0.09 4.9 (151572)
- AUU I 0.41 21.5 (668227) ACU T 0.34 17.5 (544807) AAU N 0.52 22.3 (693344) AGU S 0.16 14.0 (435738)
- 15 AUC I 0.35 18.5 (576287) ACC T 0.20 10.3 (321640) AAC N 0.48 20.9 (650826) AGC S 0.13 11.3 (352568)
- AUA I 0.24 12.6 (391867) ACA T 0.31 15.7 (487161) AAA K 0.49 30.8 (957374) AGA R 0.35 19.0 (589788)
- AUG M 1.00 24.5 (762852) ACG T 0.15 7.7 (240652) AAG K 0.51 32.7 (1016176) AGG R 0.20 11.0 (340922)
- 20 GUU V 0.40 27.2 (847061) GCU A 0.43 28.3 (880808) GAU D 0.68 36.6 (1139637) GGU G 0.34 22.2 (689891)
- GUC V 0.19 12.8 (397008) GCC A 0.16 10.3 (321500) GAC D 0.32 17.2 (535668) GGC G 0.14 9.2 (284681)
- 25 GUA V 0.15 9.9 (308605) GCA A 0.27 17.5 (543180) GAA E 0.52 34.3 (1068012) GGA G 0.37 24.2 (751489)
- GUG V 0.26 17.4 (539873) GCG A 0.14 9.0 (280804) GAG E 0.48 32.2 (1002594) GGG G 0.16 10.2 (316620)

[0076] The cell oils of this invention can be distinguished from conventional vegetable or animal triacylglycerol sources in that the sterol profile will be indicative of the host organism as distinguishable from the conventional source. Conventional sources of oil include soy, corn, sunflower, safflower, palm, palm kernel, coconut, cottonseed, canola, rape, peanut, olive, flax, tallow, lard, cocoa, shea, mango, sal, illipe, kokum, and allanblackia.

[0077] The oils provided herein are not vegetable oils. Vegetable oils are oils extracted from plants and plant seeds. Vegetable oils can be distinguished from the non-plant oils provided herein on the basis of their oil content. A variety of methods for analyzing the oil content can be employed to determine the source of the oil or whether adulteration of an oil provided herein with an oil of a different (e.g. plant) origin has occurred. The determination can be made on the basis of one or a combination of the analytical methods. These tests include but are not limited to analysis of one or more of free fatty acids, fatty acid profile, total triacylglycerol content, diacylglycerol content, peroxide values, spectroscopic properties (e.g. UV absorption), sterol profile, sterol degradation products, antioxidants (e.g. tocopherols), pigments (e.g. chlorophyll), d13C values and sensory analysis (e.g. taste, odor, and mouth feel). Many such tests have been standardized for commercial oils such as the Codex Alimentarius standards for edible fats and oils.

[0078] Sterol profile analysis is a particularly well-known method for determining the biological source of organic matter. Campesterol, b-sitosterol, and stigmasterol are common plant sterols, with b-sitosterol being a principle plant sterol. For example, b-sitosterol was found to be in greatest abundance in an analysis of certain seed oils, approximately 64% in corn, 29% in rapeseed, 64% in sunflower, 74% in cottonseed, 26% in soybean, and 79% in olive oil (Gul et al. J. Cell and Molecular Biology 5:71-79, 2006).

[0079] The sterol profile of a microalgal oil is distinct from the sterol profile of oils obtained from higher plants or animals. Oil isolated from *Prototheca moriformis* strain UTEX1435 were separately clarified (CL), refined and bleached (RB), or refined, bleached and deodorized (RBD) and were tested for sterol content according to the procedure described in JAOCS vol. 60, no.8, August 1983. Results of the analysis are shown Table 3 below (units in mg/100g):

[0080] Table 3 (units in mg/100g)

	Sterol	Crude	Clarified	Refined & bleached	Refined, bleached, & deodorized
1	Ergosterol	384 (56%)	398 (55%)	293 (50%)	302 (50%)
2	5,22-cholestadien-24-methyl-3-ol (Brassicasterol)	14.6 (2.1%)	18.8 (2.6%)	14 (2.4%)	15.2 (2.5%)
3	24-methylcholest-5-en-3-ol (Campesterol or 22,23-dihydrobrassicasterol)	10.7 (1.6%)	11.9 (1.6%)	10.9 (1.8%)	10.8 (1.8%)
4	5,22-cholestadien-24-ethyl-3-ol (Stigmasterol or poriferasterol)	57.7 (8.4%)	59.2 (8.2%)	46.8 (7.9%)	49.9 (8.3%)
5	24-ethylcholest-5-en-3-ol (β -Sitosterol or clionasterol)	9.64 (1.4%)	9.92 (1.4%)	9.26 (1.6%)	10.2 (1.7%)
6	Other sterols	209	221	216	213
	Total sterols	685.64	718.82	589.96	601.1

[0081] These results show three striking features. First, ergosterol was found to be the most abundant of all the sterols, accounting for about 50% or more of the total sterols. The amount of ergosterol is greater than that of campesterol, β -sitosterol, and stigmasterol combined. Ergosterol is steroid commonly found in fungus and not commonly found in plants, and its presence particularly in significant amounts serves as a useful marker for non-plant oils. Secondly, the oil was found to contain brassicasterol. With the exception of rapeseed oil, brassicasterol is not commonly found in plant based oils. Thirdly, less than 2% β -sitosterol was found to be present. β -sitosterol is a prominent plant sterol not commonly found in microalgae, and its presence particularly in significant amounts serves as a useful marker for oils of plant origin. In summary, *Prototheca moriformis* strain UTEX1435 has been found to contain both significant amounts of ergosterol and only trace amounts of β -sitosterol as a percentage of total sterol content. Accordingly, the ratio of ergosterol : β -sitosterol or in combination with the presence of brassicasterol can be used to distinguish this oil from plant oils.

[0082] In some embodiments, the oil content of an oil provided herein contains, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% β -sitosterol. In other embodiments the oil is free from β -sitosterol.

[0083] In some embodiments, the oil is free from one or more of β -sitosterol, campesterol, or stigmasterol. In some embodiments the oil is free from β -sitosterol, campesterol, and stigmasterol. In some embodiments the oil is free from campesterol. In some embodiments the oil is free from stigmasterol.

5 [0084] In some embodiments, the oil content of an oil provided herein comprises, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% 24-ethylcholest-5-en-3-ol. In some embodiments, the 24-ethylcholest-5-en-3-ol is clionasterol. In some embodiments, the oil content of an oil provided herein comprises, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%,
10 9%, or 10% clionasterol.

[0085] In some embodiments, the oil content of an oil provided herein contains, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% 24-methylcholest-5-en-3-ol. In some embodiments, the 24-methylcholest-5-en-3-ol is 22, 23-dihydrobrassicasterol. In some embodiments, the oil content of an oil provided
15 herein comprises, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 22,23-dihydrobrassicasterol.

[0086] In some embodiments, the oil content of an oil provided herein contains, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% 5,22-cholestadien-24-ethyl-3-ol. In some embodiments, the 5, 22-cholestadien-24-ethyl-3-ol is poriferasterol. In some embodiments, the oil content of an oil provided herein
20 comprises, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% poriferasterol.

[0087] In some embodiments, the oil content of an oil provided herein contains ergosterol or brassicasterol or a combination of the two. In some embodiments, the
25 oil content contains, as a percentage of total sterols, at least 5%, 10%, 20%, 25%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% ergosterol. In some embodiments, the oil content contains, as a percentage of total sterols, at least 25% ergosterol. In some embodiments, the oil content contains, as a percentage of total sterols, at least 40% ergosterol. In some embodiments, the oil content contains, as a percentage of total
30 sterols, at least 5%, 10%, 20%, 25%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of a combination of ergosterol and brassicasterol.

[0088] In some embodiments, the oil content contains, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, or 5% brassicasterol. In some embodiments, the oil content contains, as a percentage of total sterols less than 10%, 9%, 8%, 7%, 6%, or 5% brassicasterol.

- 5 [0089] In some embodiments the ratio of ergosterol to brassicasterol is at least 5:1, 10:1, 15:1, or 20:1.

- [0090] In some embodiments, the oil content contains, as a percentage of total sterols, at least 5%, 10%, 20%, 25%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% ergosterol and less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% β -sitosterol. In some
10 embodiments, the oil content contains, as a percentage of total sterols, at least 25% ergosterol and less than 5% β -sitosterol. In some embodiments, the oil content further comprises brassicasterol.

- [0091] Sterols contain from 27 to 29 carbon atoms (C27 to C29) and are found in all eukaryotes. Animals exclusively make C27 sterols as they lack the ability to further
15 modify the C27 sterols to produce C28 and C29 sterols. Plants however are able to synthesize C28 and C29 sterols, and C28/C29 plant sterols are often referred to as phytosterols. The sterol profile of a given plant is high in C29 sterols, and the primary sterols in plants are typically the C29 sterols β -sitosterol and stigmasterol. In contrast, the sterol profiles of non-plant organisms contain greater percentages of C27
20 and C28 sterols. For example the sterols in fungi and in many microalgae are principally C28 sterols. The sterol profile and particularly the striking predominance of C29 sterols over C28 sterols in plants has been exploited for determining the proportion of plant and marine matter in soil samples (Huang, Wen-Yen, Meinschein W. G., "Sterols as ecological indicators"; *Geochimica et Cosmochimica Acta*. Vol 43. pp 739-745).
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[0092] In some embodiments the primary sterols in the microalgal oils provided herein are sterols other than β -sitosterol and stigmasterol. In some embodiments of the microalgal oils, C29 sterols make up less than 50%, 40%, 30%, 20%, 10%, or 5% by weight of the total sterol content.

- 30 [0093] In some embodiments the microalgal oils provided herein contain C28 sterols in excess of C29 sterols. In some embodiments of the microalgal oils, C28 sterols

make up greater than 50%, 60%, 70%, 80%, 90%, or 95% by weight of the total sterol content. In some embodiments the C28 sterol is ergosterol. In some embodiments the C28 sterol is brassicasterol.

- [0094]** Where a fatty acid profile of a triglyceride (also referred to as a “triacylglyceride” or “TAG”) cell oil is given here, it will be understood that this refers to a nonfractionated sample of the storage oil extracted from the cell analyzed under conditions in which phospholipids have been removed or with an analysis method that is substantially insensitive to the fatty acids of the phospholipids (e.g. using chromatography and mass spectrometry). The oil may be subjected to an RBD process to remove phospholipids, free fatty acids and odors yet have only minor or negligible changes to the fatty acid profile of the triglycerides in the oil. Because the cells are oleaginous, in some cases the storage oil will constitute the bulk of all the TAGs in the cell. Examples 1 and 2 below give analytical methods for determining TAG fatty acid composition and regiospecific structure.
- [0095]** Broadly categorized, certain embodiments of the invention include (i) recombinant oleaginous cells that comprise an ablation of one or two or all alleles of an endogenous polynucleotide, including polynucleotides encoding lysophosphatidic acid acyltransferase (LPAAT) or (ii) cells that produce oils having low concentrations of polyunsaturated fatty acids, including cells that are auxotrophic for unsaturated fatty acids; (iii) cells producing oils having high concentrations of particular fatty acids due to expression of one or more exogenous genes encoding enzymes that transfer fatty acids to glycerol or a glycerol ester; (iv) cells producing regiospecific oils, (v) genetic constructs or cells encoding a an LPAAT, a lysophosphatidylcholine acyltransferase (LPCAT), a phosphatidylcholine diacylglycerol cholinephosphotransferase (PDCT), diacylglycerol cholinephosphotransferase (DAG-CPT) or fatty acyl elongase (FAE) , (vi) cells producing low levels of saturated fatty acids and/or high levels of C18:1, C18:2, C18:3, C20:1 or C22:1, (vii) and other inventions related to producing cell oils with altered profiles. The embodiments also encompass the oils made by such cells, the residual biomass from such cells after oil extraction, oleochemicals, fuels and food products made from the oils and methods of cultivating the cells.

[0096] In any of the embodiments below, the cells used are optionally cells having a type II fatty acid biosynthetic pathway such as plant cells, yeast cells, microalgal cells including heterotrophic or obligate heterotrophic microalgal cells, including cells classified as Chlorophyta, Trebouxiophyceae, Chlorellales, Chlorellaceae, or Chlorophyceae, or cells engineered to have a type II fatty acid biosynthetic pathway using the tools of synthetic biology (i.e., transplanting the genetic machinery for a type II fatty acid biosynthesis into an organism lacking such a pathway). Use of a host cell with a type II pathway avoids the potential for non-interaction between an exogenous acyl-ACP thioesterase or other ACP-binding enzyme and the multienzyme complex of type I cellular machinery. In specific embodiments, the cell is of the species *Prototheca moriformis*, *Prototheca krugani*, *Prototheca stagnora* or *Prototheca zopfii* or has a 23S rRNA sequence with at least 65, 70, 75, 80, 85, 90 or 95% nucleotide identity SEQ ID NO: 25. By cultivating in the dark or using an obligate heterotroph, the cell oil produced can be low in chlorophyll or other colorants. For example, the cell oil can have less than 100, 50, 10, 5, 1, 0.0.5 ppm of chlorophyll without substantial purification.

[0097] The stable carbon isotope value $\delta^{13}\text{C}$ is an expression of the ratio of $^{13}\text{C}/^{12}\text{C}$ relative to a standard (e.g. PDB, carbonite of fossil skeleton of *Belemnite americana* from Peedee formation of South Carolina). The stable carbon isotope value $\delta^{13}\text{C}$ ($^{\circ}/_{\text{oo}}$) of the oils can be related to the $\delta^{13}\text{C}$ value of the feedstock used. In some embodiments the oils are derived from oleaginous organisms heterotrophically grown on sugar derived from a C4 plant such as corn or sugarcane. In some embodiments the $\delta^{13}\text{C}$ ($^{\circ}/_{\text{oo}}$) of the oil is from -10 to -17 $^{\circ}/_{\text{oo}}$ or from -13 to -16 $^{\circ}/_{\text{oo}}$.

[0098] In specific embodiments and examples discussed below, one or more fatty acid synthesis genes (e.g., encoding an acyl-ACP thioesterase, a keto-acyl ACP synthase, an LPAAT, an LPCAT, a PDCT, a DAG-CPT, an FAE a stearoyl ACP desaturase, or others described herein) is incorporated into a microalga. It has been found that for certain microalga, a plant fatty acid synthesis gene product is functional in the absence of the corresponding plant acyl carrier protein (ACP), even when the gene product is an enzyme, such as an acyl-ACP thioesterase, that requires binding of ACP to function. Thus, optionally, the microalgal cells can utilize such genes to make a desired oil without co-expression of the plant ACP gene.

[0099] For the various embodiments of recombinant cells comprising exogenous genes or combinations of genes, it is contemplated that substitution of those genes with genes having 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or 100% nucleic acid sequence identity can give similar results, as
5 can substitution of genes encoding proteins having 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 100% amino acid sequence identity. Nucleic acids encoding the acyltransferases encode acyltransferases that have 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or
10 100% amino acid sequence identity to the acyltransferase disclosed in clade 1, clade 2, clade 3 or clade 4 of Table 5. Likewise, for novel regulatory elements, it is contemplated that substitution of those nucleic acids with nucleic acids having 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% nucleic acid can be efficacious. In the various embodiments, it will be understood
15 that sequences that are not necessary for function (e.g. FLAG® tags or inserted restriction sites) can often be omitted in use or ignored in comparing genes, proteins and variants.

[0100] The novel genes and gene combinations reported here can be used in higher plants using techniques that are well known in the art. For example, the use of
20 exogenous lipid metabolism genes in higher plants is described in U.S. Patents 6,028,247; 5,850,022; 5,639,790; 5,455,167; 5,512,482; and 5,298,421 disclose higher plants with exogenous acyl-ACP thioesterases. WO2009129582 and WO1995027791 disclose cloning of LPAAT in plants. FAD2 ablation and/or down regulation in higher plants is taught in WO 2013112578, and WO2008/006171. SAD ablation
25 and/or down regulation in higher plants is taught in WO 2013112578, and WO 2008006171.

[0101] The expression of the novel acyltransferases is shown in Examples 4, 5, 6 and 7. The expression of *Cuphea paucipetala* or *Cuphea ignea* LPATs markedly increased the C8:0 and C10:0 fraction of the cell oil. Additionally, the expression of
30 *Cuphea paucipetala* or *Cuphea ignea* LPAATs markedly increased the incorporation of C8:0 and C10:0 fatty acids in the *sn*-2 position of the TAG. This is disclosed in Example 4.

[0102] The expression of LPAT genes in host cells increased C18:2 levels and elevated the sat-unsat-sat/sat-sat-sat, (e.g., SOS/SSS) ratio of the cell oil. For example, the expression of *Theobroma cacao* LPAT2 drives the transfer of unsaturated fatty acids toward the *sn*-2 position and reduces the incorporation of saturated fatty acids at *sn*-2.

[0103] The novel LPAATs, GPATs, DGATs, LPCATs, and PLA2 with specificity for mid-chain fatty acids are disclosed. In Example 7, expression of LPAATs and DGATs are disclosed.

[0104] When an acyltransferase of the invention is expressed in a host cell, one or more additional exogenous genes can concomitantly be expressed. An embodiment of this invention provides host cells that express a recombinant acyltransferase and concomitantly express one or more additional recombinant genes. The one or more additional genes include invertase, fatty acyl-ACP thioesterase (FATA, FATB), melibiase, ketoacyl synthase (KASI, KASII, KASIII, KASIV), antibiotic selective markers, tags such as FLAG, and THIC. In Examples 4, 5, 6, and 7, the co-expression of nucleic acids that encode LPAATs co-expressed with one or more exogenous genes that encode invertase, fatty acyl-ACP thioesterase, melibiase, ketoacyl synthase, THIC are disclosed.

[0105] When an acyltransferase of the invention is expressed in a host cell, an endogenous gene of the host cell can concomitantly be ablated or downregulated, thereby eliminating or decreasing the expression of the gene of the host cell. This can be accomplished by using homologous recombination techniques or other RNA inhibitory technologies. The ablated or downregulated gene can be any gene in the host cell. The ablated or downregulated endogenous gene can be stearoyl ACP desaturase, fatty acyl desaturase, fatty acyl-ACP thioesterase (FATA or FATB), ketoacyl synthase (KASI, KASII, KASIII or KAS IV), or an acyltransferase (LPAAT, DGAT, GPAT, LPCAT). When an endogenous is ablated, one, two or more alleles of the endogenous can be ablated. In Example 5, the expression of a *Brassica* LPAAT, while concomitantly ablating an endogenous stearoyl ACP desaturase is disclosed. In Example 6, LPAATs, GPATs, DGATs, LPCATs and PLA2s with specificity for mid-chain fatty acids were expressed, while ablating a gene encoding stearoyl ACP desaturase. In Example 7 the down regulation of an endogenous FAD2 and a hairpin

RNA is disclosed. In co-owned PCT/US2016/026265, applicants disclosed concomitant ablation of an endogenous LPAAT and expression of an exogenous LPAAT.

[0106] In one embodiment, the expression of the acyl transferases alters the fatty acid profile and/or the *sn*-2 profile of the oil produced by the host organism. The fatty acid profiles and the *sn*-2 profiles that result from the expression of various acyltransferases are disclosed in Tables 6, 7, 10, 11, 12, 13, 16, 17, 18, 19, 20, 22, 23, and 24. The invention provides host cells with altered fatty acid profiles and altered *sn*-2 profiles according to Tables 6, 7, 10, 11, 12, 13, 16, 17, 18, 19, 20, 22, 23, and 24.

[0107] As described in PCT/US2016/026265, co-owned by applicant, transcript profiling was used to discover promoters that modulate expression in response to low nitrogen conditions. The promoters are useful to selectively express various genes and to alter the fatty acid composition of microbial oils. In accordance with an embodiment, there are non-natural constructs comprising a heterologous promoter and a gene, wherein the promoter comprises at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% sequence identity to any of the promoters of SEQ ID NOs: 1-18 and the gene is differentially expressed under low vs. high nitrogen conditions. In particular, the *Prototheca moriformis* AMT02 (SEQ ID NO: 18) and AMT03 promoter (SEQ ID NO: 18) are useful promoters for controlling the expression of an exogenous gene. For example, the promoters can be placed in front of a FAD2 gene in a linoleic acid auxotroph to produce an oil with less than 5, 4, 3, 2, or 1% linoleic acid after culturing first under high nitrogen conditions, then next culturing under low nitrogen conditions. Additional promoters, in particular *Prototheca* and *Chlorella* promoters are described in the sequences and descriptions in this application. For example, the *Prototheca* HXT1, SAD, LDH1 and other *Prototheca* promoters are described in Examples 6, 7, 8, and 9. Additionally, the *Chlorella* SAD, ACT and other *Chlorella* promoters are described in Examples 6, 7, 8, and 9.

[0108] In embodiments of the present invention, oleaginous cells expressing one or more of the genes encoding acyltransferases and/or variant FATA can produce an oil with at least 20, 40, 60 or 70% of C8, C10, C12, C14, C16, or C18 fatty acids.

[0109] The invention also provides host cells expressing one or more of the genes encoding acyltransferases and/or variant FATA can produce an oil enriched in oils that are sat-unsat-sat. Oils of this type include SOS, POP, POS, SLS, PLO, PLO. The sat-unsat-sat oils comprise at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%,
5 85%, 90%, or 95% of the cell oil by dry cell weight.

[0110] The invention also provides host cells expressing one or more of the genes encoding acyltransferases and/or variant FATA can produce an oil that is decreased in tri-saturated oils, sat-sat-sat. Oils of this type include PPP, PSS, PPS, SSS, SPS, and PSP. The sat-sat-sat oils comprise less than 50%, 40%, 30%, 20%, 15%, 10%, 8%,
10 6%, 5%, 4%, 3%, 2%, or 1% of the cell oil by molar fraction or dry cell weight.

[0111] The host cells of the invention can produce 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, or about 90% oil by cell weight, $\pm 5\%$. Optionally, the oils produced can be low in DHA or EPA fatty acids. For example, the oils can comprise less than 5%, 2 %, or 1% DHA and/or EPA.

15 [0112] In other embodiments of the invention, there is a process for producing an oil, triglyceride, fatty acid, or derivative of any of these, comprising transforming a cell with any of the nucleic acids discussed herein. In another embodiment, the transformed cell is cultivated to produce an oil and, optionally, the oil is extracted. Oil extracted in this way can be used to produce food, oleochemicals or other
20 products.

[0113] The oils discussed above alone or in combination are useful in the production of foods, fuels and chemicals (including plastics, foams, films, etc). The oils, triglycerides, fatty acids from the oils may be subjected to C-H activation, hydroamino methylation, methoxy-carbonation, ozonolysis, enzymatic
25 transformations, epoxidation, methylation, dimerization, thiolation, metathesis, hydro-alkylation, lactonization, or other chemical processes.

[0114] After extracting the oil, a residual biomass may be left, which may have use as a fuel, as an animal feed, or as an ingredient in paper, plastic, or other product. For example, residual biomass from heterotrophic algae can be used in such products.

30 EXAMPLES

EXAMPLE 1: FATTY ACID ANALYSIS BY FATTY ACID METHYL ESTER DETECTION

[0115] Lipid samples were prepared from dried biomass. 20-40 mg of dried biomass was resuspended in 2 mL of 5% H₂SO₄ in MeOH, and 200 µl of toluene containing an appropriate amount of a suitable internal standard (C19:0) was added. The mixture was sonicated briefly to disperse the biomass, then heated at 70 -75°C for 3.5 hours. 2 mL of heptane was added to extract the fatty acid methyl esters, followed by addition of 2 mL of 6% K₂CO₃ (aq) to neutralize the acid. The mixture was agitated vigorously, and a portion of the upper layer was transferred to a vial containing Na₂SO₄ (anhydrous) for gas chromatography analysis using standard FAME GC/FID (fatty acid methyl ester gas chromatography flame ionization detection) methods. Fatty acid profiles reported below were determined by this method.

EXAMPLE 2: ANALYSIS OF REGIOSPECIFIC PROFILE

[0116] LC/MS TAG distribution analyses were carried out using a Shimadzu Nexera ultra high performance liquid chromatography system that included a SIL-30AC autosampler, two LC-30AD pumps, a DGU-20A5 in-line degasser, and a CTO-20A column oven, coupled to a Shimadzu LCMS 8030 triple quadrupole mass spectrometer equipped with an APCI source. Data was acquired using a Q3 scan of *m/z* 350-1050 at a scan speed of 1428 u/sec in positive ion mode with the CID gas (argon) pressure set to 230 kPa. The APCI, desolvation line, and heat block temperatures were set to 300, 250, and 200°C, respectively, the flow rates of the nebulizing and drying gases were 3.0 L/min and 5.0 L/min, respectively, and the interface voltage was 4500 V. Oil samples were dissolved in dichloromethane-methanol (1:1) to a concentration of 5 mg/mL, and 0.8 µL of sample was injected onto Shimadzu Shim-pack XR-ODS III (2.2 µm, 2.0 x 200 mm) maintained at 30°C. A linear gradient from 30% dichloromethane-2-propanol (1:1)/acetonitrile to 51% dichloromethane-2-propanol (1:1)/acetonitrile over 27 minutes at 0.48 mL/min was used for chromatographic separations.

EXAMPLE 3: CULTIVATION OF MICROALGAE**Standard lipid production conditions:**

[0117] Cells scraped from a source plate with toothpicks were used to inoculate pre-seed cultures of 0.5 mL EB03, 0.5% glucose, 1X DAS2 cultures in 96-well blocks. Pre-seed cultures were grown for 70-75 h at 28°C, 900 rpm in a Multitron shaker. 40 µL of pre-seed cultures were used to inoculate seed cultures of 0.46 mL H29, 4% glucose, 25 mM citrate pH 5 or 100 mM PIPES pH 7.3, 1X DAS2 (8% inoculum), and grown for 24-28 h at 28°C, 900 rpm in a Multitron shaker. 40 µL of seed cultures were used to inoculate lipid production cultures of 0.46 mL H43, 6% glucose, 25 mM citrate pH 5, 1X DAS2 (8% inoculum), and grown for 70-75 h at 28°C, 900 rpm in a Multitron shaker. Fatty acid profiles and lipid titer analyses were performed as disclosed in Examples 1 and 2.

50 mL shake flask format

[0118] Cells scraped from a source plate with inoculation loops, or cell cultures from cryovials were used to inoculate pre-seed cultures of 10 mL EB03, 0.5% glucose, 1X DAS2 cultures in 50 mL bioreactor tubes. Pre-seed cultures were grown for 70-75 h at 28°C, 200 rpm in a Kuhner shaker. 0.8 mL of pre-seed cultures were used to inoculate seed cultures of 10 mL H29, 4% glucose, 25 mM citrate pH 5 or 100 mM PIPES pH 7.3, 1X DAS2 (8% inoculum), and grown for 24-28 h at 28°C, 200 rpm in a Kuhner shaker. 100 µL of seed cultures were used to inoculate lipid production cultures of 49.9 mL H43, 6% glucose, 25 mM citrate pH 5 or 100 mM PIPES pH 7.3, 1X DAS2 (0.2% inoculum), and grown for 118-122 h at 28°C, 200 rpm in a Kuhner shaker. Fatty acid profiles and lipid titer analyses were performed as disclosed in Examples 1 and 2.

EB03

Dry chemicals	
Component	Concentration (g/L)
K ₂ HPO ₄	3
Sodium Phosphate Dibasic Heptahydrate (Na ₂ HPO ₄ 7H ₂ O)	5.66
citric acid monohydrate	1.2
ammonium sulfate	1
MgSO ₄ 7H ₂ O	0.23
CaCl ₂ 2H ₂ O	0.03
Stock solutions	

Component	Concentration (mL/L)
100X C-Trace (3)	10
Antifoam Sigma 204	0.225

H29

Dry chemicals	Final
Component	Concentration (g/L)
K ₂ HPO ₄ (Potassium phosphate dibasic anhydrous)	0.25
NaH ₂ PO ₄ (Sodium phosphate monobasic)	0.18
MgSO ₄ ·7H ₂ O (Magnesium sulfate heptahydrate)	0.24
Citric acid monohydrate	0.25
Stock solutions	
Component	Concentration (mL/L)
0.017M stock CaCl ₂ ·2H ₂ O	10
0.151M (NH ₄) ₂ SO ₄	52.2
100X C-Trace (2)	10
Antifoam Sigma 204	0.225

H43

Dry chemicals	Final
Component	Concentration (g/L)
K ₂ HPO ₄	0.25
NaH ₂ PO ₄	0.18
MgSO ₄ 7H ₂ O	0.24
Citric acid H ₂ O	0.25
Stock solutions	
Component	Concentration (mL/L)
0.017M stock CaCl ₂ 2H ₂ O	10
100X C-Trace (2)	10
Antifoam Sigma 204	0.225
0.151M (NH ₄) ₂ SO ₄	12.5

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1000X DAS2

Dry chemicals	Final
Component	Concentration (g/L)
Thiamine-HCl	0.67
d-Biotin	0.010
Cyanocobalimin (vit B-12)	0.008
Calcium Pantothenate	0.02
PABA (p-aminobenzoic acid)	0.04

100X C-Trace(2)

Dry chemicals	Final
Component	Concentration (g/L)
CuSO4-5H2O	0.011
CoCl2-6H2O	0.081
H3BO3	0.33
ZnSO4-7H2O	1.4
MnSO4-H2O	0.81
Na2MoO4-2H2O	0.039
FeSO4-7H2O	0.11
NiCl2-6H2O	0.013
Citric Acid Monohydrate	3.0

100X C-Trace (3)

Dry chemicals	Final
Component	Concentration (g/L)
CuSO4-5H2O	0.011
H3BO3	0.33
ZnSO4-7H2O	1.4
MnSO4-H2O	0.81
Na2MoO4-2H2O	0.039
FeSO4-7H2O	0.11
NiCl2-6H2O	0.013
Citric Acid Monohydrate	3.0

EXAMPLE 4: IDENTIFICATION OF NOVEL LPAAT GENES FROM SEQUENCED TRANSCRIPTOMES AND ENGINEERING *SN-2* TAG REGIOSPECIFICITY IN UTEX1435 BY EXPRESSION OF HETEROLOGOUS LPAAT GENES FROM CUPHEA PAUCIPETALA, CUPHEA IGNEA, CUPHEA PAINTERI, AND CUPHEA HOOKERIANA.

[0119] Lysophosphatidic acyltransferase (LPAAT) genes from plant seeds were cloned and expressed in the transgenic strain, S6511, derived from UTEX 1435 (*P. moriformis*). Expression of the heterologous LPAATs increases C8:0 and C10:0 fatty acid levels and dramatically increases incorporation of C8:0 and C10:0 fatty acids at the *sn-2* position of triacylglycerols (TAGs) in transgenic strains.

[0120] TAGs are synthesized from various chain length acyl-CoAs and glycerol-3-phosphate by consecutive action of three ER-resident enzymes of the Kennedy pathway - glycerol phosphate acyltransferase (GPAT), LPAAT, and diacylglycerol acyltransferase (DGAT). Substrate specificities of these acyltransferases are known to determine the fatty acid composition of the resulting TAGs. LPAAT acylates the *sn-2* hydroxyl group of lysophosphatidic acid (LPA) to form phosphatidic acid (PA), a precursor to TAG. In co-owned applications WO2013/158938, WO2015/051139, and PCT/US2016/026265 we demonstrated expression of LPAAT from *Cocos nucifera* (*CnLPAAT*, accession no. AAC49119; Knutzon et al., 1995).

[0121] Strain S6511 expresses the acyl-ACP thioesterase (*FATB2*) gene from *Cuphea hookeriana* (*ChFATB2*), leading to C8:0 and C10:0 fatty acid accumulation of ca. 14% and 28%, respectively. Strain S6511 is a strain made according to the methods disclosed in co-owned WO2010/063031 and WO2010/063032, herein incorporated by reference. Briefly, S6511 is a strain that express sucrose invertase and a *C. hookeriana* *FATB2*. The construct pSZ3101: 6S::CrTUB2-ScSUC2-CvNR_a:PmAMT03-CpSAD1tp_trimmed:ChFATB2-CvNR_d::6S was engineered into S3150, a strain classically mutagenized to increase lipid yield. We identified novel C8:0- and C10:0-specific *LPAATs* from seeds exhibiting high levels of C8:0 and C10:0 fatty acids. After we identified and cloned LPAATs we expressed the *LPAAT* genes in S6511.

Method for Identification of LPAATs

[0122] Seeds were obtained from species exhibiting elevated levels of midchain and other specialized fatty acids (Table 4).

[0123] Table 4: Fatty acid profiles of mature seeds. The percentage of each fatty acid making up the seed oil is shown; abundant and unusual fatty acid species are indicated in bold.

		C8 :0	C10: 0	C12: 0	C1 4:0	C1 6:0	C1 8:0	C1 8:1 (p etr as eli na te)	C1 8:2	C2 0:0	C2 0:1	C2 2:0	C2 2:1 n1 7	C22: 1n9	C2 2:2 n9 17	C2 2: 2n 6
S01_C c	Cinnamom um camphora	0.4	54.7	39.0	1.6	0.7	0.1	2.9		0.6	0.0					
S02_Uc	Umbellulari a californica	0.9	28.8	63.0	2.3	0.4	0.1	3.4		0.6	0.0					
S03_L d	Limnanthes douglasii	0.0	0.0	0.0	0.4	0.7	0.4	2.7		1.5	1.5	59.9	0.3	2.8	17.4	9.3 0.5
S04_C hs	Cuphea hyssopifoli a	0.2	6.5	83.7	5.1	1.1	0.1	0.0		1.7	0.1					
S05_C cr	Cuphea carthagene nsis	1.6	8.1	59.2	15.2	3.9	0.6	0.0		5.4	0.2					
S06_C pr	Cuphea parsonsia	2.0	11.5	61.3	10.8	2.7	0.5	0.0		5.2	0.1					
S07_C g	Cuphea glossostom a	7.1	85.1	1.7	0.3	1.0	0.2	0.0		2.1	0.1					
S08_C ht	Cuphea heterophyll a	3.5	44.3	40.0	4.3	1.2	0.3	2.2		3.6	0.1					
S11_ Dc	Daucus carrota	0.0	0.0	0.0	0.1	5.9	0.8	11.5	65.9	13.0	0.5	0.3	0.3			
S14_C w	Cuphea wrightii	0.5	20.2	62.5	5.8	2.2	0.3	2.7		4.7						
S15_B j	Brassica juncea	0.0	0.0	0.0	0.1	3.2	0.7	12.1		19.2	0.5	6.3	0.8		38.9	1.3
S16_B r	Brassica rapa nipposinica	0.0	0.0	0.0	0.1	2.8	1.0	16.0		16.8	0.7	8.3	1.0		40.4	0.8
S17_C a	Cuphea avigera var. pulcherrim a	90.8	2.7	0.0	0.1	1.2	0.1	1.8		2.8						
S18_C h	Cuphea hookeriana	64.7	29.7	0.1	0.2	1.3	0.1	1.9		2.0						
S19_C pal	Cuphea palustris	28.9	0.8	1.3	55.1	6.2	0.2	3.0		3.4						
S20_C pai	Cuphea painteri	67.0	20.8	0.1	0.2	2.6	0.3	3.1		4.5						
S21_C pau	Cuphea paucipetala	1.5	91.0	1.2	0.7	1.5	0.2	1.1		2.1						
S22_C hook	Cuphea hookeriana	62.8	31.9	0.2	0.2	1.0	0.1	2.1		1.2						
S23_C glut	Cuphea glutinosa	5.2	29.9	46.4	3.9	1.9	0.4	0.0		8.1						
S24_C aequ	Cuphea aequipetal a	27.1	0.0	1.4	57.4	6.0	0.2	3.2		3.8						
S25_C calc	Cuphea calcarata	8.0	20.4	46.8	7.6	3.2	0.6	3.7		8.5						
S26_C hook	Cuphea hookeriana	70.4	23.1	0.1	0.2	1.5	0.2	2.5		1.8						
S27_C proc	Cuphea procumben s	0.9	86.3	0.0	1.6	2.2	0.4	3.2		3.3						
S28_C ignea	Cuphea ignea	3.1	84.9	0.7	0.3	2.6	0.2	2.9		4.4						
S35_C cras	Cuphea crassiflora	1.3	87.7	1.3	0.4	2.0	0.5	3.3		2.7						

S36_C koe	Cuphea koehneana	0.0	87.4	1.4	0.8	2.2	0.4	2.3		4.5						
S37_C lept	Cuphea leptopoda	1.3	86.1	1.3	0.4	2.2	0.5	3.1		4.1						
S40_C lop	Cuphea lophostoma	0.5	82.3	2.4	1.6	3.0	0.6	3.9		4.9						
S41_S al	Sassafras albidum db	4. 3	65.2	22.8	0.9	0.8	5.1	0.0		0.6						

- [0124] Briefly, RNA was extracted from dried plant seeds and submitted for paired-end sequencing using the Illumina Hiseq 2000 platform. RNA sequence reads were assembled into corresponding seed transcriptomes using the Trinity software package.
- 5 LPAAT-containing cDNA contigs were identified by mining transcriptomes for sequences with homology to a known LPAAT that was previously identified in-house, *CuPSR23 LPAAT2-1* (seeWO2013/158938), using BLAST. For some sequences, a high-confidence, full-length transcript was assembled using Trinity. The resulting amino acid sequences of all new LPAATs were subjected to phylogenetic analyses
- 10 using previously known, full-length LPAAT sequences (available via NCBI) as well as sequences of previously known LPAATs whose sequences were derived at Solazyme. The analysis showed that the amino acid sequences of the newly discovered LPAATs were not similar to previously known LPAATs. Table 5 shows the clade analysis in which the novel LPAATs were clustered according to a neighbor
- 15 joining algorithm. These were found to form 4 clades as listed in Table 5.

[0125] Table 5: Clade Analysis of LPAATs

Clade No.	Amino Acid SEQ ID Nos. in Clade	Full Genus Species	Function	Percent amino acid identity to members of clade
1	S15 BjLPAAT1d	<i>Brassica juncea</i>		96.3
	S15 BjLPAAT1c	<i>Brassica juncea</i>		
	S15 BjLPAAT1a	<i>Brassica juncea</i>		
	S15 BjLPAAT1b	<i>Brassica juncea</i>		
2	CuPSR23LPAAT2-1	<i>Cuphea PSR23</i>	Prefer C8 / C10 sn-2	93.9
	S40 CloLPAAT1	<i>Cuphea lophostoma</i>		
	S21 CpauLPAAT1	<i>Cuphea paucipetala</i>		
	S37 CleptLPAAT1	<i>Cuphea leptopoda</i>		
	S27 CprocLPAAT1b	<i>Cuphea procumbens</i>		
	S27 CprocLPAAT1	<i>Cuphea procumbens</i>		
	S04 ChsLPAAT2	<i>Cuphea hyssopifolia</i>		

	S28 CignealPAAT1	<i>Cuphea ignea</i>		
	S05 CcrLPAAT2a	<i>Cuphea carthagenensis</i>		
	S06 CprLPAAT1	<i>Cuphea parsonsia</i>		
	S05 CcrLPAAT2b	<i>Cuphea carthagenensis</i>		
	S17 CalPAAT3	<i>Cuphea avigera</i> var. <i>pulcherrima</i>		
	S26 ChookLPAAT1	<i>Cuphea hookeriana</i>		
	S20 CpaiLPAAT1	<i>Cuphea painteri</i>		
	S04 ChsLPAAT1	<i>Cuphea hyssopifolia</i>		
	S25 Ccalc1a	<i>Cuphea calcarata</i>		
	S25 Ccalc1b	<i>Cuphea calcarata</i>		
	S14 CwLPAAT1	<i>Cuphea wrightii</i>		
	S08 ChtLPAAT1a	<i>Cuphea heterophylla</i>		
	S08 ChtLPAAT1b	<i>Cuphea heterophylla</i>		
	S36 CkoeLPAAT2	<i>Cuphea koehneana</i>		
	S02 UclPAAT1b	<i>Umbellularia californica</i>		
	S02 UclPAAT1a	<i>Umbellularia californica</i>		
	S01 CcLPAAT1a	<i>Cinnamomum camphora</i>		
	S01 CcLPAAT1b	<i>Cinnamomum camphora</i>		
	S41 SalLPAAT1	<i>Sassafras albidum</i> db		
3	S14 CwLPAAT2a	<i>Cuphea wrightii</i>	C18:2	86.5
	S14 CwLPAAT2b	<i>Cuphea wrightii</i>		
	S25 CcalcLPAAT2	<i>Cuphea calcarata</i>		
	S19 CpallPAAT1	<i>Cuphea palustris</i>		
	S22 ChookLPAAT3b	<i>Cuphea hookeriana</i>		
	S17 CalPAAT1	<i>Cuphea avigera</i> var. <i>pulcherrima</i>		
	S22 ChookLPAAT3a	<i>Cuphea hookeriana</i>		
	CuPSR23LPAAT3-1	<i>Cuphea PSR23</i>		
	S27 CprocLPAAT2b	<i>Cuphea procumbens</i>		
	S27 CprocLPAAT2a	<i>Cuphea procumbens</i>		
	S18 ChLPAAT2a	<i>Cuphea hookeriana</i>		
	S24 CaequLPAAT1d	<i>Cuphea aequipetala</i>		
	S24 CaequLPAAT1b	<i>Cuphea aequipetala</i>		

	S24 CaequLPAAT1a	<i>Cuphea aequipetala</i>		
	S24 CaequLPAAT1c	<i>Cuphea aequipetala</i>		
	S23 CglutLPAAT1a	<i>Cuphea glutinosa</i>		
	S23 CglutLPAAT1b	<i>Cuphea glutinosa</i>		
	S26 ChookLPAAT2b	<i>Cuphea hookeriana</i>		
	S07 CgLPAAT1c	<i>Cuphea glossostoma</i>		
	S07 CgLPAAT1b	<i>Cuphea glossostoma</i>		
	S07 CgLPAAT1a	<i>Cuphea glossostoma</i>		
	S28 CignealLPAAT2	<i>Cuphea ignea</i>		
	S36 CkoeLPAAT1	<i>Cuphea koehneana</i>		
	S35 CcrasLPAAT1a	<i>Cuphea crassiflora</i>		
	S35 CcrasLPAAT1c	<i>Cuphea crassiflora</i>		
	S35 CcrasLPAAT1b	<i>Cuphea crassiflora</i>		
	S35 CcrasLPAAT1d	<i>Cuphea crassiflora</i>		
4	Gh LPAAT2B	<i>Garcinia hombroriana</i>	Reduced trisaturates, increase unsaturates at Sn-2 position	78.5
	Gi LPAAT2B-1	<i>Garcinia indica</i>		
	Gh LPAAT2A	<i>Garcinia hombroriana</i>		
	Gi LPAAT2A	<i>Garcinia indica</i>		
	Gh LPAAT2C	<i>Garcinia hombroriana</i>		
	Gi LPAAT2C-2	<i>Garcinia indica</i>		
	S03 LdLPAAT1	<i>Limnanthes douglasii</i>		
	S11 DcLPAAT1	<i>Daucus carota</i> (carrot)		
	S11 DcLPAAT2	<i>Daucus carota</i> (carrot)		
	S11 DcLPAAT2 (truncated)	<i>Daucus carota</i> (carrot)		

Functionality of LPAATs in *P. moriformis*

[0126] To increase the levels of C8:0 and C10:0 fatty acids in strain S6511, as well as to test the functionality of the newly identified LPAATs, we identified midchain-specific LPAATs from the transcriptomes of species exhibiting high levels of C8:0 and C10:0 fatty acids in their oil seeds and introduced the genes into S6511. LPAATs that co-clustered with *CuPSR23 LPAAT2-1*, specifically *CpauLPAAT1*, *CignealLPAAT1*, *ChookLPAAT1*, and *CpaiLPAAT1*, were selected for synthesis and testing. *CpauLPAAT1*, *CignealLPAAT1*, *ChookLPAAT1*, and *CpaiLPAAT1* were

synthesized in a codon-optimized form to reflect UTEX 1435 codon usage.

Transgenic strains were generated via transformation of the strain S6511 with a construct encoding one of the four LPAAT genes. The construct pSZ3840 encoding CpauLPAAT1 is shown as an example, but identical methods were used to generate

- 5 each of the remaining three constructs. Construct pSZ3840 can be written as pLOOP::PmHXT1-ScarMEL1-CvNR:PmAMT3-CpauLPAAT1-CvNR::pLOOP. The sequence of the transforming DNA is provided in Figure 2 (pSZ3840). The relevant restriction sites in the construct from 5'-3', BspQI, KpnI, SpeI, XhoI, EcoRI, SpeI, XhoI, SacI, BspQI, respectively, are indicated in lowercase, bold, and underlined.
- 10 BspQI sites delimit the 5' and 3' ends of the transforming DNA. Bold lowercase sequences at the 5' and 3' end of the construct represent genomic DNA from UTEX 1435 that target integration to the pLOOP locus via homologous recombination.
- Proceeding in the 5' to 3' direction, the selection cassette has the *P. moriformis* HXT1 promoter driving expression of the *Saccharomyces carlsbergensis* MEL1 (conferring
- 15 the ability to grow on melibiose) and the *Chlorella vulgaris* Nitrate reductase (NR) gene 3' UTR. The promoter is indicated by lowercase, boxed text. The initiator ATG and terminator TGA for *ScarMEL1* are indicated in bold, uppercase italics, while the coding region is indicated with lowercase italics. The 3' UTR is indicated by lowercase underlined text. The second cassette containing the codon optimized
- 20 *CpauLPAAT1* gene from *Cuphea paucipetala* is driven by the *P. moriformis* AMT3 promoter and has the *Chlorella vulgaris* Nitrate reductase (NR) gene 3' UTR. In this cassette, the *AMT3* promoter is indicated by lowercase, boxed text. The initiator ATG and terminator TGA for the *CpauLPAAT1* gene are indicated in bold, uppercase italics, while the coding region is indicated by lowercase italics. The 3' UTR is
- 25 indicated by lowercase underlined text. The final construct was sequenced to ensure correct reading frame and targeting sequences.

[0127] SEQ ID NO: 19 pSZ3840/D2554 transforming construct (*CpauLPAAT1*)

30 gctcttccgctaacggaggtctgtcaccaaatggacccgcttattgcgggaaaccacggcgatggcacgtttcaaaac
ttgatgaaatacaatattcagtatgtcgcgggcgacggcgaggagctgatgtcgcgctgggtattgcttaatcgcc
agcttcgccccgctcttggtgagggcgtgaacaagccgaccgatgtgcacgagcaaactctgacactagaagggtg
actcgcggcgacggctgaattacacaggcttcaaaaataccagaatttcacgcaccgtattcgcggtattttgttg
acagtgaatagcgatgcggcaatggcttgtggcgttagaaggtgcgacgaaggtggtgccaccactgtgccagccagt
cctggcggtccaggggcccgatcaagagccaggacatccaaactaccacagcatcaacgccccggcctatactcg
35 aacccacttgactctgcaatggtatgggaaccacggggcagtcctgtgtgggtcgcgcctatcgcggtcggcgaaga

cgggaaggtaccgcggtgagaatcgaaaatgcatcgttttaggttcggagacgggtcaattccctgctccggcgaatct
 gtcggtcaagctggccagtggacaatgttgctatggcagcccgcgacatgggctcccgcgagccatcaggagccc
 aaacagcgtgtcagggtatgtgaaactcaagaggtccctgctgggactccggcccaactccggggcgggacgcca
 gcattcgcggtcgggtcccgcgacgagcgaaatgatgattcggttacgagaccaggacgtcgtcgaggtcgagaggc
 5 agcctcggacacgtctcgtagggcaacgcccaggtcccgcgagggccgtaaacattgtttctgggtgtcggagtgg
 gcattttgggcccgatccaatcgctcatgcccgtctcgtctggtcctcacgttcgctgacggcctggatcccggaaaggg
 cggatgcacgtggtgttccccgccattggcgccacgtttcaaagtccccggccagaaatgcacaggaccggcccgcc
 tcgcacaggccatgctgaacgccagatttcgacagcaacacatctagaataatcgcaacatccgcgtttgaacga
 aacgaaacggcgctgttttagcatgtttccgacatcgtggggccgaagcatgtccggggggaggaaagcgtggcaca
 10 gcggtagccattctgtgccacgcccagaggaccaatccccggcatcagccttcacgagcgtgcgcgcacata
 taaagccggacgcctaaccgggttcgtggttatgactagtATGttcgcgttctacttctgacggcctgcatctccctga
 agggcgtgttcggcgtctcccctctacaacggcctgggctgacgcccagatgggctgggacaactggaacac
 gttcgcctgcgacgtctccgagcagctgctgctggacacggccgaccgcatctccgacctgggctgaaggacatgg
 gctacaagtacatcatctggacgactgctggtcctcggccgcgactccgacggcttctggtcggcagcagcag
 15 aagttcccaacggcatgggccacgtcgcgaccacctgcacaacaactccttctggttcggcatgtactcctccgcg
 ggcgagtacacgtgcgcggctaccccggtccctgggcccgcgagggaggagcggccagtcttcgcgaacaac
 cgcgtggactacctgaagtacgacaactgctacaacaagggccagttcggcagccccgagatctctaccaccgt
 acaaggccatgtccgacgcccgaacaagacgggcccgcacatcttctactccctgtgcaactggggccaggacctg
 accttctactggggtcggcatcggaactcctggcgatgtccggcgacgtcacggcgagttcacgcgccccga
 20 ctcccgctgcccctgcgacggcgacgagtacgactgcaagtacgcccgttccactgctccatcatgaacatcctga
 acaaggccgcccccatgggcccagaacgcggcgctcggcggtggaacgacctggacaacctggaggtcggcgtc
 ggcaacctgacggacgacgaggagaaggcgacttctccatgtgggcatgtgtaagtccccctgatcatcggc
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 aacggcatccccgccacgcgctgtggcgctactacgtgtccgacacggacgagtacggccaggcgagatccag
 25 atgtggtcggccccctggacaacggcgaccaggtcgtggcgctgctgaacggcggtccgtgtccgccccatga
 acacgacctggaggagatcttcttcgactccaacctgggtccaagaagctgacctccacctgggacatctacgac
 ctgtgggcgaaccgcgtcgacaactccacggcgtccgccatcctgggcccgaacaagaccgccaccggcatcctgt
 acaacgccaccgagcagtcctacaaggacggcctgtccaagaacgacaccgcctgttcggccagaagatcggct
 cctgtcccccaacgcgatctgaacacgaccgtccccgccacggcatcgcttctaccgcctgcgcccctcctcTG
 30 Atacgtactcaggcagcagcagctcggatagtatcgacacactctggacgctggtcgtgtgatggactgttccgccac
 acttgccttgacctgtgaatatccctgcccgttttatcaaacagcctcagtggtttgatcttgtgtgtacgcgctttgc
 gagttgctagctgcttgtgctatttgcgaataaccacccccagcatccccttccctcgttcatatcgcttgcacccaaccgc
 aacttatctacgtgtcctgctatccctcagcgtgctcctgctcactgcccctgcacagccttggtttgggctcc
 gcctgtattctcctggtactgcaacctgtaaacagcactgcaatgctgatgcacgggaagtagtgggatgggaacaca
 35 aatggaaagctgtgaattcggccgacaggacgcgctcaaaggtgctggtcgtgtatgccctggccggcaggtcgttg
 ctgctgctggttagtgattccgaaccctgattttggcgcttattttggcggtggcaacgctggcgcccgagccgggc
 cggcggtgatgcggtccccacggctgccggaatccaaggaggcaagagcggccgggtcagttgaagggtttacgc
 gcaaggtacagccgctcctgcaaggctgcgtggtggaattggacgtgcaggtcctgctgaagttcctccaccgctcacc
 agcggacaaagcaccggtgtatcaggtccgtgtcatcactctaagagctcgactacgacctactgatggccctagatt
 40 cttcatcaaaaacgctgagacacttcccaggattgaaactccctgaagggaaccaccagggggccctgagttgttccttc
 ccccggtggcgagctgccagccaggctgtacctgtgatcgaggctggcgggaaaataggcttcgtgtgctcaggtcatg
 ggaggtgcaggacagctcatgaaacccaacaatcgcaaatcatgtcaagctaatacgtatttctcttcacgagct
 gtaattgtccaaaattctggtctaccgggggtgatccttcgtgtacgggcccctccctcaaccctaggtatgcgcgcatg
 cggctcgcgcgcaactcgcgagggccgaggggttgggacgggcccgtcccgaatgcagttgcacccggatgcgtgg

caccttttttgcgataatttatgcaatggactgctctgcaaaattctggctctgtcgccaaccctaggatcagcggttagg
 atttcgtaatcattcgtcctgatggggagctaccgactaccctaataatcagcccagctgctgacgccagcgtccacttttg
 tgcacacattccattcgtgccaagacatttcattgtggtgcgaagcgtcccagttacgctcacctgtttcccgacctcct
 tactgttctgtcgacagagcgggccacaggccggtcgagccactagtATGgccatccccccgcccgcctggtgatt
 5 cctgttcggcctgctgttttccactccggcctgatcatcaacctgttccaggccctgtgcttcgtgctggtgtggccct
 gtccaagaacgcctaccgcccatcaaccgcgtgttcgccgagctgctgctgtccgagctgctgtgctgttcgactg
 gtggggccggcgccaagctgaagctgttcaccgaccccagaccttccgcctgatgggcaaggagcacgccttggtg
 atcatcaaccacatgaccgagctggactggatgctgggctgggtgatgggccagcacctgggtgctgggtcca
 tctgttcgtggccaagaagtccaccaagttcctgcccgtgctgggctggtccatgtggttctccgagtacctgtacat
 10 cgagcgtcctgggccaaggaccgacccacctgaagtccacatcgagcgcctgaccgactacccctgccccttct
 ggatggtgatcttcgtggagggcaccgccttaccgcaccaagctgctggccgcccagcagtacgccgcctcctcc
 ggctgcccgtgccccgaacgtgctgatccccgcaccaagggcttctgtgctgctgtccacatgcgctccttcg
 tgcggccgtgtacgacgtgaccgtggccttccccaaagacctccccccccccacctgctgaacctgttcgagggcc
 agtccatcgtgctgcacgtgcacatcaagcgccacgcatgaaggacctgcccagctccgacgacgcctggccca
 15 gtggtgccgacgaagttcgtggagaaggacgcctgctggacaagcacaacgccgaggacaccttctccggcca
 ggaggtgcaccgcaccggctccgccccatcaagtcctgctggtggtgatctcctgggtggtggtgatcaccttcgg
 cgccctgaagttctcgcagtgttctcctggaagggaaggccttctcctgatcggcctgggcatcgtgacctgct
 gatgcacatgctgatcctgtcctcccaggccgagcgtcctccaaccccgccaaggtggcccaggccaagctgaag
 accgagctgtccatctccaagaaggccaccgacaaggagaacTGActcgaggcagcagcagctcgatagtatcg
 20 acacactctggacgctggtcgtgtgtaggactgttgccgacacattgctgccttgacctgtgaatatcctgcccgtttat
caaacagcctcagtggttgatcttgtgtgtacgcgcttttgcgagttgtagctgctgtgtgctatttgcgaataaccaccc
cagcatccccttccctcgttccatcgttgcacccaaccgcaacttatctacgctgctcgtatccctcagcgctgctc
ctgctcctgctcactgcccctcgacagccttgggttgggctccgctgtattctcctggtactgcaacctgtaaacagca
ctgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggaaagcttgagctcagcgcgacggtcctgcta
 25 ccgtacgacgttgggcacgcccataaaagttgtataccgagcttgttgagcgaactgcaagcgcggtcaaggatact
tgaactcctggattgatcgggtccaataatggatggaaaatccgaacctcgtgcaagaactgagcaaacctcgttac
atggatgcacagtcgccagtcacatgaacattgaagtgcgaactgttcgcttcggtggcagtactactcaaagaat
gagctgctgttaaaaatgcactctcgttctcctaagtgagtgagcagatgagtgctcacgccttgacttcgctgcccgtgt
catgccctgcgccccaaaattgaaaaaagggtgagattattgggcaatggacgacgtcgtcgtccgggagtcagg
 30 accggcggaataaagaggcaacacactccgcttcttagctcttc

[0128] The sequence for all of the other LPAAT constructs are identical to that of
 pSZ3840 with the exception of the encoded LPAAT. The LPAAT sequence alone
 with flanking SpeI and XhoI restriction sites is provided for the remaining LPAAT
 35 constructs are shown below. The amino acid sequence of the LPAAT proteins is
 provided below.

[0129] SEQ ID NO: 30 pSZ3841/D2555 (*CpaiLPAAT1*)
actagtATGgccatccccccgcccgcctggtgttctgttcggcctgctgttttccactccggcctgatcatcaacct
 gttccaggccttctgcttcgtgctgatctccccctgtccaagaacgcctaccgcccatcaaccgcgtgttcgccgag
 40 ctgctgcccctggagttcctgtggctgttcactggtgcgccggcgccaagctgaagctgttcaccgaccccagacc
 ttcgcctgatgggcaaggagcacgccttggtgatcatcaaccacaagatcgagctggactggatggtgggctgg
 gtgctgggccagcacctgggtgctgggtccatcctgtccgtggccaagaagtccaccaagttcctgcccgtgttc

ggctggccctgtggttctccggctacctgttcttgagcgctcctgggccaaggacaagatcacctgaagtccac
 atcgagtcctgaaggactacccctgcccttctggctgatcatcttctggaggggcaccgcttcacccgcaccaag
 ctgctggccgcccagcagtagccgctcctccggcctgcccgtgcccgcacacgtgctgatccccacaccaagggc
 ttcgtgtcctccgtgtcccacatgcgctccttctgctcccgccatctacgacgtgaccgtggccttccccaagacctcccc
 5 cccccaccatgctgaagctgttcgagggccagtcctgaggctgcacgtgcacatcaagcgccacgccatgaagg
 acctgcccagagtcgacgacgccgtggccagtggtgcccgcacaagttcgtggagaaggacgccctgctggaca
 agcacaactccgaggacaccttctccggccaggaggtgcaccacgtggccgccccatcaaggccctgctggtggt
 gatctcctgggtggtggtgatcatcttcggcgccctgaagttcctgctgtggtcctcctgctgtcctcctggaagggc
 aaggccttctccgtgatcgccctgggcatcgtggccggcatcgtgacctgctgatgcacatcctgatcctgtcctcc
 10 aggccgagggctccaaccccgtaaggccgccccgccaagctgaagaccgagctgtcctcctcaagaaggtga
 ccaacaaggagaac**TGActcgag**

[0130] SEQ ID NO: 21 pSZ3842/D2556 (CignealLPAAT1)

actagtATGgccatcgccgcccgcgcctgatcttctgttcggcctgctgttcttcgctccggcatcatcatcaacct
 15 gttccaggccctgtgcttctgctgatctggccctgtccaagaacgtgtaccgccgcatcaaccgctgttcgccga
 gctgctgctgatggacctgctgtgcctgttcactggtgggcccggcgaagatcaagctgttcaccgaccccgaga
 ccttcgcctgatgggcatggagcagccctggtgatcatgaaccacaagaccgacctggactggatggtgggctg
 gatcctgggcccagcacctgggctgcctgggctccatcctgtccatcgccaagaagtccaccaagttcatccccgtgct
 gggctggtccgtgtggttctccgagtacctgttctggagcgctcctgggccaaggacaagttcacccctgaagtccca
 20 catggagaagctgaaggactacccctgcccttctggctggtgatcttctgaggggcaccgcttcacccgcacca
 agctgctggccgcccagcagtagccgctcctccggcctgcccgtgcccgcacacgtgctgatccccacaccaag
 ggcttcgtgtcctgctgtccaacatgcgctccttctgctgcccgcctgtacgacgtgaccgtggccttcccaagtctc
 cccccccccaccatgctgaagctgttcgagggccagtcctcgtgctgcacgtgcacatcaagcgccacgcccctga
 aggacctgcccagagtcgacgacgccgtggccagtggtgcccgcacaagttcgtggagaaggacgccctgctgg
 25 acaagcacaacgccgaggacaccttctccggccaggaggtgcaccacatcgccgccccatcaagtcctgctggt
 ggtgatcgctgggtggtggtgatcatcttcggcgccctgaagttcctgcagtggtcctcctgctgtccacctggaag
 ggcaaggccttctccgtgatcgccctgggcatcgccacctgctgatgcacatgctgatcctgtcctccaggccgag
 cgctccaaccccgccaaggtggccaag**TGActcgag**

[0131] SEQ ID NO: 22 pSZ3844/D2557 (ChookLPAAT1)

actagtATGgccatccccctccgcccgcctggtgttctgttcggcctgctgttcttcacctccggcctgatcatcaacct
 gttccaggccttctgcttctgctgatctccccctgtccaagaacgcctaccgccgcatcaaccgctgttcgccgag
 ctgctgcccctggagttctgtggtgttcactggtgcggcgccgaagctgaagctgttcaccgaccccgagacc
 ttcgcctgatgggcaaggagcagccctggtgatcatcaaccacaagatcgagctggactggatggtgggctgg
 35 gtgctgggcccagcacctgggctgctgggctccatcctgtccgtggccaagaagtcaccaagttcctgcccgtgttc
 ggctggtccctgtggttctccgagtacctgttctggagcgctcctgggccaaggacaagatcacctgaagtccac
 atcgagtcctgaaggactacccctgcccttctggctgatcatcttctggaggggcaccgcttcacccgcaccaag
 ctgctggccgcccagcagtagccgctcctccggcctgcccgtgcccgcacacgtgctgatccccacaccaagggc
 ttcgtgtcctccgtgtcccacatgcgctccttctgctcccgccatctacgacgtgaccgtggccttccccaagacctcccc
 40 cccccaccatgctgaagctgttcgagggccagtcctgaggctgcacgtgcacatcaagcgccacgccatgaagg
 acctgcccagagtcgacgacgccgtggccagtggtgcccgcacaagttcgtggagaaggacgccctgctggaca
 agcacaactccgaggacaccttctccggccaggaggtgcaccacgtggccgccccatcaaggccctgctggtggt
 gatctcctgggtggtggtgatcatcttcggcgccctgaagttcctgctgtggtcctcctgctgtcctcctggaagggc
 aaggccttctccgtgatcgccctgggcatcgtggccggcatcgtgacctgctgatgcacatcctgatcctgtcctcc
 45 aggccgagggctccaaccccgtaaggccgccccgccaagctgaagaccgagctgtcctcctcaagaaggtga
 ccaacaaggagaac**TGActcgag**

[0132] To determine the impact of the *CpauLPAAT1*, *CigneaLPAAT1*, *ChookLPAAT1*, and *CpaiLPAAT1* genes on mid-chain fatty acid accumulation, the above constructs containing the codon optimized *CpauLPAAT1*, *CigneaLPAAT1*, *ChookLPAAT1*, and *CpaiLPAAT1* genes were transformed into strain S6511. Primary transformants were clonally purified and grown under standard lipid production conditions at pH 7.0 (all the strains require growth at pH 7.0 to allow for maximal expression of the *LPAAT* gene driven by the pH-regulated *AMT3* promoter). The resulting profiles from a set of representative clones arising from these transformations are shown in Table 6.

[0133] **Table 6:** Transformants of pSZ3840 (*CpauLPAAT1*), pSZ3841 (*CpaiLPAAT1*), pSZ3842 (*CigneaLPAAT1*), and pSZ3844 (*ChookLPAAT1*). The fatty acid profiles for transgenic strains expressing LPAATs derived from *C. paucipetala*, *C. painteri*, *C. ignea*, and *C. hookeriana*.

Sample ID		C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 a
Parent	S6511a	14.4	27.7	0.6	1.3	8.8	1.6	38.2	5.4	0.4
	S6511b	14.5	27.7	0.6	1.3	8.6	1.6	38.4	5.3	0.4
pSZ3840 <i>CpauLPAAT1</i>	S6511; T792; D2554-20	16.6	29.9	0.7	1.3	8.0	1.0	35.2	5.2	0.5
	S6511; T792; D2554-17	14.6	28.7	0.6	1.3	8.4	1.7	37.1	5.7	0.5
	S6511; T792; D2554-41	15.2	28.5	0.7	1.3	8.3	1.4	37.5	5.2	0.4
	S6511; T792; D2554-35	14.7	28.4	0.6	1.3	8.6	1.6	37.3	5.6	0.5
	S6511; T792; D2554-27	15.2	27.6	0.7	1.3	9.5	1.5	37.1	5.1	0.4
pSZ3841 <i>CpaiLPAAT1</i>	S6511; T792; D2555-34	17.3	29.5	0.7	1.3	7.8	1.2	35.1	5.1	0.4
	S6511; T792; D2555-43	17.5	29.1	0.7	1.3	8.0	0.9	35.4	5.0	0.5
	S6511; T792; D2555-10	15.7	28.3	0.7	1.3	8.6	1.6	36.2	5.7	0.5
	S6511; T792; D2555-22	16.0	27.9	0.7	1.3	8.4	0.9	37.8	5.0	0.4
	S6511; T792; D2555-44	15.3	27.5	0.6	1.3	8.1	1.8	38.2	5.4	0.4
pSZ3842 <i>CigneaLPAAT1</i>	S6511; T792; D2556-38	16.2	29.2	0.7	1.3	8.1	1.3	36.1	5.2	0.5
	S6511; T792; D2556-22	14.3	28.5	0.7	1.3	8.5	1.6	37.6	5.7	0.5
	S6511; T792; D2556-44	13.6	28.4	0.7	1.4	9.0	1.5	36.3	6.7	0.7
	S6511; T792; D2556-14	14.1	28.0	0.6	1.3	8.6	1.7	38.0	5.6	0.5
	S6511; T792; D2556-36	14.3	28.0	0.6	1.3	8.6	1.7	37.9	5.7	0.5
pSZ3844 <i>ChookLPAAT1</i>	S6511; T792; D2557-47	15.8	29.3	0.7	1.3	8.2	1.2	36.5	5.0	0.5
	S6511; T792; D2557-24	16.8	28.8	0.7	1.3	8.1	1.2	35.8	5.4	0.5
	S6511; T792; D2557-30	15.2	28.3	0.7	1.3	8.5	1.6	36.8	5.7	0.5
	S6511; T792; D2557-39	14.7	28.2	0.7	1.3	8.7	1.5	37.3	5.7	0.5
	S6511; T792; D2557-26	15.3	27.7	0.7	1.4	8.7	0.9	37.7	5.4	0.5

[0134] The transformants in Table 6 display a marked increase in the production of C8:0 and C10:0 fatty acids upon expression of the heterologous LPAATs. To determine if expression of the heterologous LPAAT genes affected the regiospecificity of fatty acids at the *sn*-2 position, we analyzed TAGs from representative D2554 (*CpauLPAAT1*), D2555 (*CpaiLPAAT1*), D2556 (*CignealLPAAT1*), and D2557 (*ChookLPAAT1*) strains utilizing the porcine pancreatic lipase method. Cells were grown under conditions to maximize midchain fatty acid levels and to generate sufficient biomass for TAG analysis. TAG and *sn*-2 profiles are shown in Table 7.

[0135] **Table 7:** Inclusion of C8:0 and C10:0 fatty acids at the *sn*-2 position of TAGs. Selected transformants were subjected to porcine pancreatic lipase determination of fatty acid inclusion at the *sn*-2 position. The general fatty acid distribution in triacylglycerols (TAG) is shown to indicate fatty acid abundance for each transformant. In addition, the *sn*-2-specific distribution is shown. Numbers highlighted in bold and italic reflect significantly increased inclusion of the noted fatty acid compared to the parent S6511.

Table 7

Strain:		S6511		S6511; T792; D2554-20 (<i>CpauLPAAT1</i>)		S6511; T792; D2555-34 (<i>CpaiLPAAT1</i>)		S6511; T792; D2556-38 (<i>CignealLPAAT1</i>)		S6511; T792; D2557-24 (<i>ChookLPAAT1</i>)	
Analysis		TAG	<i>sn</i> -2	TAG	<i>sn</i> -2	TAG	<i>sn</i> -2	TAG	<i>sn</i> -2	TAG	<i>sn</i> -2
Fatty Acid (area %)	C8:0	14.4	8.5	16.6	12.8	17.3	22.3	16.2	10.0	16.8	29.1
	C10:0	27.7	26.4	29.9	39.0	29.5	22.2	29.2	36.2	28.8	19.4
	C12:0	0.6	0.4	0.7	0.3	0.7	0.4	0.7	0.4	0.7	0.3
	C14:0	1.3	1.0	1.3	1.0	1.3	0.9	1.3	1.2	1.3	0.9
	C16:0	8.8	0.9	8.0	1.1	7.8	1.1	8.1	1.2	8.1	0.9
	C18:0	1.6	0.2	1.0	0.4	1.2	0.5	1.3	0.5	1.2	0.3
	C18:1	38.2	52.5	35.2	37.8	35.1	43.6	36.1	42.2	35.8	40.7
	C18:2	5.4	8.9	5.2	6.2	5.1	7.9	5.2	7.0	5.4	7.1
	C18:3 α	0.4	0.8	0.5	0.7	0.4	0.9	0.5	0.8	0.5	0.7
	C8+C10 sum	42.2	34.9	46.4	51.8	46.8	44.5	45.5	46.1	45.6	48.5

[0136] As disclosed in Table 7, the *CpauLPAAT1* and *CignealLPAAT1* genes show remarkable specificity towards C10:0 fatty acids. D2554-20 exhibits 39.0% of C10:0

in the *sn*-2 position versus just 26.4% in the S6511 base strain without the heterologous LPAAT, demonstrating a 1.5 fold increase in C10:0 inclusion at the *sn*-2 position. D2556-38 exhibits 36.2% of C10:0 in the *sn*-2 position versus 26.4% in the S6511 base strain, demonstrating a 1.4 fold increase in C10:0 inclusion at the *sn*-2 position. Although there is a small increase in C8:0 levels in the D2554-20 and D2555-34 strains, the vast majority of *sn*-2 targeting is C10:0-specific. Similarly, *CpaiLPAAT1* and *ChookLPAAT1* show remarkable specificity towards C8:0 fatty acids. D2555-34 exhibits 22.3% C8:0 in the *sn*-2 position versus just 8.5% in the S6511 base strain without the heterologous LPAAT, demonstrating a 2.6 fold increase in C8:0 inclusion at the *sn*-2 position. D2557-24 exhibits 29.1% C8:0 in the *sn*-2 position versus 8.5%, demonstrating a 3.4 fold increase in C8:0 inclusion at the *sn*-2 position. We teach that *CpauLPAAT1* and *CignealLPAAT1* are C10:0-specific LPAATs and that *CpaiLPAAT1* and *ChookLPAAT1* are C8:0-specific LPAATs. Knutzon DS, Lardizabal KD, Nelsen JS, Bleibaum JL, Davies HM, Metz JG (1995) Cloning of a coconut endosperm cDNA encoding a 1-acyl-sn-glycerol-3-phosphate acyltransferase that accepts medium-chain-length substrates. Plant Physiol 109:999–1006

Amino acid sequences for novel LPAAT genes

[0137] SEQ ID NO: 23 *CpauLPAAT1*

MAIPAAAVIFLFGLLFFTSGLIINLFQALCFVLVWPLSKNAYRRINRVFAELLLS
ELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMLGWVMG
QHLGCLGSILSVAKKSTKFLPVLGWSMWFSEYLYIERSWAKDRITTLKSHIERL
TDYPLPFWMVIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLPRTKGFVSCV
SHMRSFVPAVYDVTVAFPKTSPPPTLLNLFEGQSIVLHVHIKRHAMKDLPESD
DAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHRTGSRPIKSLLVVISWVV
VITFGALKFLQWSSWKGKAFSVIGLGIVTLLMHMLILSSQAERSSNPAKVAQA
KLKTELSISKATDKEN

[0138] SEQ ID NO: 24 *CprocLPAAT1*

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPISKNAYRRINRVFAELLLSE
LLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMGQ
HFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWNKDKSTLKSHIERLK
DYPLPFWLVIFAEGTRFTQTKLLAAQQYAASSGLPVPRNVLPRTKGFVSCVS
HMRSFVPAVYDLTVAFPKTSPPPTLLNLFEGQSVVLHVHIKRHAMKDLPESD
DEVAQWCRDKFVEKDALLDKHNAEDTFSGQELQHTGRRPIKSLLVVISWVV
VIAFGALKFLQWSSWKGKAFSVIGLGIVTLLMHMLILSSQAERSKPAKVAQA
KLKTELSISKTVTDKEN

[0139] SEQ ID NO: 25 *CprocLPAAT1b*

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPISKNA YRRINRVFAELLSE
 LLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMGQ
 HFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWNKDKSTLKSHIERLK
 DYPLPFWLVIFAEGTRFTQTKLLAAQQYAASSGLPVPRNVLPRTKGFVSCVS
 5 HMRSFVPAVYDLTVAFPKTSPPTLLNLFEGQSVVLHVHIKRHAMKDLPESD
 DEVAQWCRDKFVEK

[0140] SEQ ID NO: 26 CprocLPAAT2a

IVNLVQAVCFVLVRPLSKNTYRRINRVVAELLWLELVWLIDWWAGVKIKVFT
 DHETFHLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKF
 10 LPVIGWSMWFSEYLFERNWAKDESTLKSGLNRLKDYPLPFWLALFVEGTRF
 TRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSFVPAIYDVTVAIP
 KTSPPTLIRMFKGQSSVLHVHLKRHVMMKDLPESDDAVAQWCRDIFVEKDAL
 LDKHNADDTFSGQELQDTGRPIKSLLVVISWAVLEVFGAVKFLQWSSLLSSW
 KGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPAKAKIEGESSKTEMEKEK

[0141] SEQ ID NO: 27 CprocLPAAT2b

IVNLVQAVCFVLVRPLSKNTYRRINRVVAELLWLELVWLIDWWAGVKIKVFT
 DHETFHLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKF
 LPVIGWSMWFSEYLFERNWAKDESTLKSGLNRLKDYPLPFWLALFVEGTRF
 TRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSFVPAIYDVTVAIP
 20 KTSPPTLIRMFKGQSSVLHVHLKRHVMMKDLPESDDAVAQWCRDIFVEKDAL
 LDKHNADDTFSGQELQDTGRPIKSLLV

[0142] SEQ ID NO: 28 CpaiLPAAT1

MAIPSAAVVFLFGLLFFTSGLIINLFQAFCFVLISPLSKNA YRRINRVFAELLPLE
 FLWLFHWCAGAKLKLFTDPETFRLMGKEHALVIINHKIELDWMVGWVLGQH
 25 LGCLGSILSVAKKSTKFLPVFGWSLWFSGYLFLERSWAKDKITLKSHIESLKD
 YPLPFWLIIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLPHTKGFVSSVSHM
 RSFVPAIYDVTVAFPKTSPPTMLKLFEGQSVELHVHIKRHAMKDLPESDDAV
 AQWCRDKFVEKDALLDKHNSEDTFSGQEVHHVGRPIKALLVVISWVVVIIFG
 ALKFLWSSLLSSWKGAFSVIGLGIVAGIVTLLMHILILSSQAEGSNPVKAAP
 30 AKLKTLSKKVTNKEN

[0143] SEQ ID NO: 29 ChookLPAAT1

MAIPSAAVVFLFGLLFFTSGLIINLFQAFCFVLISPLSKNA YRRINRVFAELLPLE
 FLWLFHWCAGAKLKLFTDPETFRLMGKEHALVIINHKIELDWMVGWVLGQH
 LGCLGSILSVAKKSTKFLPVFGWSLWFSGYLFLERSWAKDKITLKSHIESLKD
 35 YPLPFWLIIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLPHTKGFVSSVSHM
 RSFVPAIYDVTVAFPKTSPPTMLKLFEGQSVELHVHIKRHAMKDLPESDDAV
 AQWCRDKFVEKDALLDKHNSEDTFSGQEVHHVGRPIKALLVVISWVVVIIFG
 ALKFLWSSLLSSWKGAFSVIGLGIVAGIVTLLMHILILSSQAEGSNPVKAAP
 AKLKTLSKKVTNKEN

[0144] SEQ ID NO: 30 ChookLPAAT2a

LSLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRINRVVAELLWLELVWLIDWW
 AGVKIKVFTDHETFNLMGKEHALVVCNHKSDIDWLVGWVLAQRSGCLGSTL
 AVMKKSSKFLPVIGWSMWFSEYLFERSWAKDESTLKSGLKRLKDYPLPFW
 LALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSFVP

AIYDVTVAIPKTSVPPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVAQWC
RDIFVEKDALLDKHNAEDTFSGQELQDIGRPIKSLLVVISWAVLVIFGAVKFLQ
WSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKNEGESS
KTEMEKEH

5 **[0145] SEQ ID NO: 31 ChookLPAAT2b**

QIKVFTDHETFNLMGKEHALVVCNHKSDIDWLVGWVLAQWSGCLGSTLAV
MKKSSKFLPVIGWSMWFSEYLFLEERSWAKDESTLKSGLKRLKDYPLPFWLAL
FVEGTRFTQAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSFVPAIY
DVTVAIPKTSVPPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDI
10 FVEKDALLDKHNAEDTFSGQELQDIGRPIKSLLVVISWAVLVIFGAVKFLQWS
SLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKLKKEGESSKPE
TDKQN

[0146] SEQ ID NO: 32 ChookLPAAT3a

LSLLFFVSGLIVNLVQAVCFVLIRPLLKNTYRRINRVVAELLWLELVWLIDWW
15 AGIKIKVFTDHETFHLMGKEHALVICNHKSDIDWLVGWVLAQMSGCLGSTLA
VMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKSGLNRLKDYPLPFWL
ALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSQMRSFVPA
IYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHLMNDLPESDDDAVAQWCR
DIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWATLVVFGAVKFLQ
20 WSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPAKPKNEGESSK
TEMEKEH

[0147] SEQ ID NO: 33 ChookLPAAT3b

LSLLFFVSGLIVNLVQAVCFVLIRPLLKNTYRRINRVVAELLWLELVWLIDWW
AGIKIKVFTDHETFHLMGKEHALVICNHKSDIDWLVGWVLAQMSGCLGSTLA
25 VMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKSGLNRLKDYPLPFWL
ALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSQMRSFVPA
IYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHLMNDLPESDDDAVAQWCR
DIFVEKDALLDKHNAEDTFSGQELQDIGRPIKSLLVVISWAVLEIFGAVKFLQ
WSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKEGESS
30 KPETDKEN

[0148] SEQ ID NO: 34 CigneaLPAAT1

MAIAAAAVIFLFGLLFFASGIIINLFQALCFVLIWPLSKNVYRRINRVFAELLLM
DLLCLFHHWAGAKIKLFTDPETFRLMGMEHALVIMNHKTDLDWMVGWILG
QHLGCLGSILSIKAKSTKFIPVLGWSVWFSEYLFLEERSWAKDKSTLKSHMEKL
35 KDYPLPFWLVIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLPHTKGFVSCV
SNMRSFVPAVYDVTVAFPKSSPPPTMLKLFEGQSIVLHVHIKRHALKDLPESD
DAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHHIGRPIKSLLVVIWVVI
IFGALKFLQWSSLLSTWKGKAFSVIGLGIATLLMHMLILSSQAERSNPAKVAK

[0149] SEQ ID NO: 35 CigneaLPAAT2

40 MAIAAAAVIFLFGLLFFASGIIINLFQALCFVLIWPLSKNVYRRINRVFAELLLM
DLLCLFHHWAGAKIKLFTDPETFRLMGMEHALVIMNHKTDLDWMVGWILG
QHLGCLGSILSIKAKSTKFIPVLGWSVWFSEYLFLEERSWAKDESTLKSGLNRL
KDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPKNVLPRTKGFVSSV
SHMRSFVPAIYDVTVAIPKTSAPPTLLRMFKGQSSVLHVHLKRHLMKDLPESD

DAVAQWCRDIFVEKDALLDKHNAEDTFSGQELHDIGRPVKSLLVVISWAML
VVFQAVKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAP
AKQKNNEGESSKTEMEKEH

[0150] SEQ ID NO: 36 DcLPAAT1

5 SGLVVNLIQAFFFLVLRPFSKNAYRKINRVVAELLWLELIWLIDWWAGVKIQ
LYTDPETFKLMGKEHALVICNHKSDIDWLVGWILAQRSGCLGSALAVMKSS
KFLPVIGWSMWFSEYLFLEERSWAKDENTLKSGFQRLRDFPHAFWLALFVEGT
RFTQAKLLAAQEYASSMGLPAPRNVLIPRTKGFVTAVTHMRPFVPAVYDVT
AIPKTSPPPTMLRLFKGQSSVVHILKRHLMSDLPKSDDSVAQWCKDAFVVK
10 DNLLDKHKENDSFGDGVLDQDTGRPLNSLVVISWACLLIFGALKFFQWSSILS
SWKGLAFSAVGLGIVTVLMQILIQFSQSERSNRPMPKSHAK

[0151] SEQ ID NO: 37 DcLPAAT2

MAIPTAAYVVPLGAIFFFSGLLVNLIQAFFFITVWPLSKKTYIRINKVIVELLWL
EFVWLADWWAGLKIEVYADAETFQLMGKEHALVICNHKSDIDWLVGWILA
15 QRAGCLGSSFAVTKKSARYLPVVGWSIWFSGAIFLERSWEKDENTLKAGFQR
LREFPCAFWLGLFVEGTRFTQAKLLAAQEYASTMGLPFPRNVLPRTKGFIAA
VNHMREFVPAIYDLTFAFPKDSPPPTMLRLKQPSVVHVHVKRHLMKDLPE
KNEAVAQWCKDVLVKDKLLDKHKDDGSFGDGELHEIGRPLKSLVVVTTW
ACLLILGTLKFLWSSLLSSWKGLIFSATGLAVLTVLMQFLIQSTQSERSNPAS
20 LSK

[0152] SEQ ID NO: 38 CcrLPAAT1a

LGLLFFISGLAVNLIQAVCFVFLRPLSKNTYRKINRVLAELLWLQLVWLVDW
WAGVKIKVFADRESFNLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSS
LAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDESTLKEGLRRLKDFPRPFW
25 LALFVEGTRFTQAKLLAAQEYATSQGLPVPRNVLPRTKVHVHVHVKRHLMKEL
PETDEAVAQWCKDLFVEKDKLLDKHVAEDTFSDQPLQDIGRPVKPLLVS
WACLVAYGALKFLQWSSLLSSWKGLIAVSAVALAIVTILMQIMILFSQSERSIPA
KVA

[0153] SEQ ID NO: 39 CcrLPAAT1b

30 LGLLFFISGLAVNLIQAVCFVFLRPLSKNTYRKINRVLAELLWLQLVWLVDW
WAGVKIKVFADRESFNLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSS
LAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDESTLKEGLRRLKDFPRPFW
LALFVEGTRFTQAKLLAAQEYATSQGLPVPRNVLPRTKGFVSAVSHMRSFVP
AVYDMTVAIPKSSPSPTMLRLFKGQSSVVHVHVKRHLMKELPETDEAVAQW
35 CKDLFVEKDKLLDKHVAEDTFSDQPLQDIGRPVKPLLVS
WACLVAYGALKFLQWSSLLSSWKGLIAVSAVALAIVTILMQIMILFSQSERSIPTKVA

[0154] SEQ ID NO: 40 CcrLPAAT2a

MAIAAAAVVFLFGLLFFTSGLIINLAQAVCFVLIWPLSKNAYRRINRVFAELL
 LELLWLFHWRAKAKLKLADPETFRLFGKEHALVICNHRTDLDWMVGWVL
 GQHFGLGSLSVAKKSTKFLPVLGWSMWFSEYLFLEERSWAKDKSTLKSHT
 5 RLKDYPLPFWLGFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKLHVH
 IKRYAMKDLPESSDAVAQWCRDIYVEKDAFLDKHNAEDTFSGQEVHHIGRPI
 KSLLVVISWVVIIFGALKFLRWSSLLSSWKGAFSVIGLGIVTLLVNILILSSQ
 AERSNPAKVAPAKLKTLSKKTEN

[0155] SEQ ID NO: 41 CcrLPAAT2b

MAIAAAAVVFLFGLLFFTSGLIINLAQAVCFVLIWPLSKNAYRRINRVFAELL
 LELLWLFHWRAKAKLKLADPETFRLFGKEHALVICNHRTDLDWMVGWVL
 GQHFGLGSLSVAKKSTKFLPVLGWSMWFSEYLFLEERSWAKDKSTLKSHT
 RLKDYPLPFWLGFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKGFVS
 SSMHMRSFVPAVYDLTVAFPKTSPPTLLKLFEGQSVVLHVHIKRYAMKDL
 10 ESDDAVAQWCRDIYVEKDAFLDKHNAEDTFSGQEVHHIGRPIKSLLVVISWV
 VIIFGALKFLRWSSLLSSWKGAFSVIGLGIVTLLVNILILSSQAERSNPAKVA
 PAKLKTLSKKTEN

[0156] SEQ ID NO: 42 BrLPAAT1a

AAAVIVPLGILFFISGLVVNLLQAICYVLIRPLSKNTYRKINRVVAETLWLELV
 20 WIVDWWAGVKIQVFADNETFNRMGKEHALVVCNHRSDIDWLVGWILAQRS
 GCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKSGLQRLN
 DFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELVPRNVLPRTKGFVSAVS
 NMRSFVPAIYDMTVAIPKTSPPTMLRLFKGQPSVVHVHIKCHSMKDLPESSD
 AIAQWCRDQFVAKDALLDKHIAADTFPGQQEQNIGRPIKSLAVVLSWSCLLIL
 25 GAMKFLHWSNLFSSWKGIASFALGLGIITLCMQILIRSSQSERSTPAKVPAKP
 KDNHNDSGSSSQTE

[0157] SEQ ID NO: 43 BrLPAAT1b

AAAVIVPLGILFFISGLVVNLLQAVCYVLRPMSKNTYRKINRVVAETLWLELV
 VWIVDWWAGVKIQVFADNETFNRMGKEHALVVCNHRSDIDWLVGWILAQR
 30 SGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKSGLQRL
 NDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELVPRNVLPRTKGFVSAV
 SNMRSFVPAIYDMTVAIPKTSPPTMLRLFKGQPSVVHVHIKCHSMKDLPESSD
 DAIAQWCRDQFVAKDALLDKHIAADTFPGQQEQNIGRPIKSLAVVLSWSCLLI
 LGAMKFLHWSNLFSSWKGIASFALGLGIITLCMQILIRSSQSERSTPAKVPAK
 35 PKDNHNDSGSSSQTE

[0158] SEQ ID NO: 44 BrLPAAT1c

MAIAAAVIVPLGLLFFISGLLMNLLQAICYVLRPLSKNTYRKINRVVAETLW
 LELVWIVDWWAGVKIKVFADNETFSRMGKEHALVVCNHRSDIDWLVGWIL
 AQRSGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKSGL
 40 QRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELVPRNVLPRTKGFV
 SAVSNMRSFVPAIYDMTVAIPKTSPPTMLRLFKGQPSVVHVHIKCHSMKDL
 ESDDAIAQWCRDQFVAKDALLDKHIAADTFPGQQEQNIGRPIKSLAVVLSWS
 CLLILGAMKFLHWSNLFSSWKGIASFALGLGIITLCMQILIRSSQSERSTPAKV
 VPAKPKDNHNDSGSSSQTE

[0159] SEQ ID NO: 45 BjLPAAT1a

INLVVAETLWLELVWIVDWWAGVKIQVFADDETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLERNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELPVPR
 5 NVLIPRTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHV
 HIKCHSMKDLPESDDAIAQWCRDQFVAKDALLDKHIAADTFPGQKEQNIGRP
 IKSLAVSLIKTFPWLHPHQLTNIFVLFQVVVSWACLLTLGAMKFLHWSNLFSS
 WKGIALSAFGLGIITLCMQILIRSSQSERSTPAKVAPAKPK

[0160] SEQ ID NO: 46 BjLPAAT1b

10 INLVVAETLWLELVWIVDWWAGVKIQVFADDETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLERNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELPVPR
 NVLIPRTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHV
 HIKCHSMKDLPEPEDEIAQWCRDQFVAKDALLDKHIAADTFPGQKEQNIGRPI
 15 KSLAVVSWACLLTLGAMKFLHWSNLFSSWKGIALSAFGLGIITLCMQILIRSSQSERSTPAKVAPAKPK

[0161] SEQ ID NO: 47 BjLPAAT1c

INLVVAETLWLELVWIVDWWAGVKIQVFADDETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLERNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELPVPR
 20 NVLIPRTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHV
 HIKCHSMKDLPESDDAIAQWCRDQFVAKDALLDKHIAADTFPGQKEQNIGRP
 IKSLAVVLSWSCLLILGAMKFLHWSNLFSSWKGIAFSALGLGIITLCMQILIRSSQSERSTPAKVVPAPKPDNHNDSGSSSQTE

[0162] SEQ ID NO: 48 BjLPAAT1d

INLVVAETLWLELVWIVDWWAGVKIQVFADDETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLERNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELPVPR
 NVLIPRTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHV
 30 HIKCHSMKDLPESDDAIAQWCRDQFVAKDALLDKHIAADTFPGQKEQNIGRP
 IKSLAVSLS

[0163] SEQ ID NO: 49 CcLPAAT1a

MAIGVAAIVVPLGLLFILSGLMVNLIQAICFILVRPLSKNMYRRVNRVVVELLWLELIWLIDWWGGVKVDVYADSETFQSLGKEHALVVSNHRSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYVFLERSWAKDESTLKSGLRRLKDFPRPFWLALFVEGTRFTQAKLLAAREYAASTGLPIPRNVLIPRTKGFV
 35 SAVSNMRSFVPAIYDVTVAIPKTQPSPTMLRIFNRQPSVVHVHIKRRHSMNQLPQTDEGVGQWCKDIFVAKDALLDRHLAE

[0164] SEQ ID NO: 50 CcLPAAT1b

40 MAIGVAAIVVPLGLLFILSGLMVNLIQAICFILVRPLSKNMYRRVNRVVVELLWLELIWLIDWWGGVKVDVYADSETFQSLGKEHALVVSNHRSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYVFLERSWAKDESTLKSGLRRLKDFPRPFWLALFVEGTRFTQAKLLAAREYAASTGLPIPRNVLIPRTKGFV
 SAVSNMRSFVPAIYDVTVAIPKTQPSPTMLRIFNRQPSVVHVHIKRRHSMNQLP

QTDEGVAQWCKDIFVAKDALLDRHLAEGKFDEKEFKRIRRPKISLLVISSWSF
LLMFGVFKFLKWSALLSTWKGVAVSTTVLLLVTVVMYMFILFSQSERSSPRK
VAPSGPENG

[0165] SEQ ID NO: 51 UcLPAAT1a

5 MAIGVAAIVVPLGLLFILSGLIINLIQAICFILVRPLSKNMYRKVNRVVVELLWL
ELIWLIDWWGGVKVDVYADSETFQSLGKEHALVVSNNHRSDIDWLVGWVLA
QRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYVFLERSWAKDESTLKSGLQR
LKDFPRPFWLALFVEGTRFTQAKLLAAQEYAASTGLPIPRNVLIPTKGFVSA
VSNMRSFVPAIYDVTVAIPKTQPSPTMLRIFNRQPSVVHVHIKRHSMNQLPQT
10 DEGVAQWCKDIFVAKDALLDRHLAEGKFDEKEFKLIRRPKISLLVISSWSFLL
MFGVFKFLKWSALLSTWKGVAVSTAVLLLVTVVMYMFILFSQSERSSPRKV
APIGPENG

[0166] SEQ ID NO: 52 UcLPAAT1b

MAIGVAAIVVPLGLLFILSGLIINLIQAICFILVRPLSKNMYRKVNRVVVELLWL
15 ELIWLIDWWGGVKVDVYADSETFQSLGKEHALVVSNNHRSDIDWLVGWVLA
QRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYVFLERSWAKDESTLKSGLQR
LKDFPRPFWLALFVEGTRFTQAKLLAAQEYAASTGLPIPRNVLIPTKGFVSA
VSNMRSFVPAIYDVTVAIPKTQPSPTMLRIFNRQPSVVHVHIKRHSMNQLPQT
DEGVAQWCKDIFVAKDALLDRHLAE

20 **[0167] SEQ ID NO: 53 LdLPAAT1**

SLLFFMSGVLVNFQAVFYVLVRPISKNTYRRINTLVAELLWLELVWVIDWW
AGVKVQLYTDTESFRLMGKEHALLICNHRSDIDWLIGWVLAQRCGCLSSSIA
VMKKSSKFLPVIGWSMWFSEYLFLEARNWAKDENTLKSGLQRLNDFPKPFWL
ALFVEGTRFTKAKLLAAQEYAAAGLPVPRNVLIPTKGFVSAVSNMRSFVP
25 AIYDLTVAIPKTTEQPTMLRLFRGKSSVVHVHLKRHLMKDLPKTDGVAQW
CKDQFISKDALLDKHVAEDTFSGLEVQDIGRPMKSLVVVSWMCLLCLGLV
KFLQWSALLSSWKGMMITTFVLGIVTVLMHILIRSSQSEHSTPAK

[0168] SEQ ID NO: 54 CaequLPAAT1a

QRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEARNWAKDESTLKSGLKR
30 LKDYPLPFWLALFVEGTRFTQAKLLAAQQAASSGLPVPRNVLIPTKGFVSS
VSHMRSFVPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPES
DDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLVVISWAVL
VIFGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAP
AKPKKEGESSKTETOKEN

35 **[0169] SEQ ID NO: 55 CaequLPAAT1b**

DWWAGVKIKVFTDHETLSLMGKEHALVISNHSKDIDWLVGWVLAQRSGCL
GSTLAVMKKSSKFLPVIGWSMWFSEYLFLEARNWAKDESTLKSGLKRLKDYPL
PFWLALFVEGTRFTQAKLLAAQQAASSGLPVPRNVLIPTKGFVSSVSHMR
SFVPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVA
40 QWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLV

[0170] SEQ ID NO: 56 CaequLPAAT1c

DWWAGVKIKVFTDHETLSLMGKEHALVISNHSKDIDWLVGWVLAQRSGCL
GSTLAVMKKSSKFLPVIGWSMWFSEYLFLEARNWAKDESTLKSGLKRLKDYPL
PFWLALFVEGTRFTQAKLLAAQQAASSGLPVPRNVLIPTKGFVSSVSHMR

SFVPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESSDDAVA
QWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLVVISWAVLVIFGA
VKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKK
EGESSKTETOKEN

5 [0171] SEQ ID NO: 57 **CaequLPAAT1d**

QRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYFLERSWAKDESTLKSGLKRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLPRTKGFVSS
VSHMRSFVPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESSDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLV

10 [0172] SEQ ID NO: 58 **CglutLPAAT1a**

LSLLFFVSGLFVNLVQAVCFVLIRPFSKNTYRRINRVVAELLWLELVWLIDW
WAGVKKIKVFTDHETLSLMGKEHALVISNHKSDIDWLVGWVLAQRSGCLGST
LAVMKKSSKFLPVIGWSMWFSEYFLERSWAKDESTLKSGLKRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSF
VPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESSDDAVAQ
WCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLVVISWAVLVIFGAV
KFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKE
GESSKTETOKEN

[0173] SEQ ID NO: 59 **CglutLPAAT1b**

20 QAVCFVLIRPFSKNTYRRINRVVAELLWLELVWLIDWWAGVKKIKVFTDHETL
SLMGKEHALVISNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIG
WSMWFSEYFLERSWAKDESTLKSGLKRLKDYPLPFWLALFVEGTRFTQAK
LLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSFVPAIYDVTVAIPKMST
PPTMLRIFKGQSSVLHVHLKRHLMKDLPESSDDAVAQWCRDIFVEKDALLDK
25 HNAEDTFSGQELQDIGRPVKSLLVVISWAVLVIFGAVKFLQWSSLLSSWKGL
AFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKEGESSKTETOKEN

[0174] SEQ ID NO: 60 **CprLPAAT1**

MAIAAAAVVFLFGLLFFTSGLIINLAQAVCFVLIWPLSKNAYRRINRVFAELL
LELLWLFHWRAGAKLKLFADPETFRLFGKEHALVICNHRDLDWMVGWVL
30 GQHFGLGSLSVAKKSTKFLPVLGWSMWFSEYFLERSWAKDKSTLKSHT
RLKDYPLPFWLGIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKGFVS
SMHMRSFVPAVYDLTVAFPKTSPPTLLKLFEGQSVVLHVHIKRYAMKDL
ESDDAVAQWCRDIYVEKDAFLDKHNAEDTFSGQEVHIGRPIKSLLVVISWV
VVIIFGALKFLRWSSLLSSWKGAFSVIGLGIVTLLVNILILSSQAERSNPAKVV
35 PAKLKTTELSPSKKVTNKEN

[0175] SEQ ID NO: 61 **ChsLPAAT1**

MAIPSAAVVFLFGLLFFASGLIINLVQAVCFVLIWPLSKNTCRRINIVFQDMLL
SELLWLFHWRAGAKLKFFTDPEYRHMKGKEHALVITNHRDLDWMIGWVL
GEHLGCLGSLSVVKKSTKFLPVLGWSMWFSEYFLERNWAKDKSTFKSHIE
40 RLEDFPQPFWFGIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKGFVS
SVSHMRSFVPAVYETMTTFPKTSPPTLLKLFEGQPLVLIHMKRHAMKDIPE
SDDAVAQWCRDKFVEKDALLDKHNAEDTFGGLEVHIGRSIKSLMVVICWV
VIIFGALKFLQWSSLLSSWKGIAGIFIGLGIVNLLVHVLILSSQAERSAPTKVAP
AKLKTLLSSKKITNKEN

[0176] SEQ ID NO: 62 ChsLPAAT2

MAIPSAAVVFLFGLLFFASGLIINLVQAVCFVLIWPLSKNTCRRINIVFQDMLL
 SELLWLFHWRAGAKLKFFTDPEYRHMKGKEHALVITNHRDLDWMIGWVL
 GEHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYLFLERNWAKDKSTFKSHIE
 5 RLEDFPQPFWFVGFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSS
 VSHMRSFVPAIYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPE
 SDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPIKSLVVVISWAA
 LVVFGAVKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAP
 AKPKREGESSKTEMEN

10 [0177] SEQ ID NO: 63 CcalcLPAAT1a

MAIPAAAVVFLFGLLFFPSGLIINLFQAVCFVLIWPFSRNTCRRINIVFQEMLLS
 ELLWLFHWRAGAKLKLFADPEYRHMKGKEHALLITNHRDLDWMIGWALG
 QHLGCLGSILSVVKKSTKFLPSHIERLEDFPQPFWMAIFVEGTRFTRAKLLAAQ
 QYAASSGLPVPRNVLPRTKGFVSCVSHMRSFVPAVYETTMTPKTSPPPTLL
 15 KLFEGQPIVLHVHMKRHAMKDIPESDEAVAQWCRDKFVEKDSLLDKHNAGD
 TFSCQEIHIGRPIKSLMVVISWVVVIFGALKFLQWSSLLSSWKGLAFSGIGLGI
 TLLVHILILSSQAERSTPAKVAPAKLKTLSSTKVTNKEN

[0178] SEQ ID NO: 64 CcalcLPAAT1b

MAIPAAAVVFLFGLLFFPSGLIINLFQAVCFVLIWPFSRNTCRRINIVFQEMLLS
 20 ELLWLFHWRAGAKLKLFADPEYRHMKGKEHALLITNHRDLDWMIGWALG
 QHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYLFLERNWAKDKSTFKSHIER
 LEDFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSC
 VSHMRSFVPAVYETTMTPKTSPPPTLLKLFEGQPIVLHVHMKRHAMKDIPES
 DEAVAQWCRDKFVEKDSLLDKHNAGDTFSCQEIHIGRPIKSLMVVISWVVVII
 25 FGALKFLQWSSLLSSWKGLAFSGIGLGIIVTLLVHILILSSQAERSTPAKVAPAKL
 KTELSSSTKVTNKEN

[0179] SEQ ID NO: 65 CcalcLPAAT2

LSLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRINRVVAELLWLELVWLIDWW
 AGVKIKVFTDHFRLMGTEHALVISNHKSDIDWLVGWVLAQRSGCLGSTLA
 30 VMKKSSKFLPVIGWSMWFSEYLFLERSWAKDESTLKSGLNRLKDYPLPFWL
 ALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSFVPA
 IYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPESSDDAVAQWC
 RDIFVEKDALLDKHNAEDTFSGQELQDIGRPIKSLVVVISWAALVVFGAVKFL
 QWSSLLSSWKGLAFSGIALGIITLLMHILILFSQSERSTPAKVAPAKPKKEGESS
 35 KTETDKEN

[0180] SEQ ID NO: 66 ChLPAAT1a

MAIPAAAVIFLFSILFFASGLIINLVQAVCFVLIWPLSKNTCRRINLVFQEMLLS
 ELLGLFHWRAKAKLKLYTDPETYPLLGKEHALLMINHRDLDWMIGWVLGQ
 HLGCLGSILSVVKKSTKFLPVLGWSMWFSEYLFLERNWAKDKSTFKSHIERLE
 40 DFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKGFVSTVS
 HMRSFVPAVYDTTLTFPKTSPPPTLLNLFAGQPIVLHIHIKRHAMKDIPESDDA
 VAQWCRDKFVEKDALLDKHNAEDAFSDQEFPIRSIKSLMVVISWVMVIFG
 ALKFLQWSSLLSSWKGLAFSVIAGVIVTLLMHMSILSSQAERSNPAKVALPKL
 KTELPSSKKVLNKEN

[0181] SEQ ID NO: 67 ChtLPAAT1b

MAIPAAAVIFLFSILFFASGLIINLVQAVCFVLIWPLSKNTCRRINLVFQEMLLS
 ELLGLFHWRAKAKLKYTDPETYPLLGKEHALLMINHRTDLDWMIGWVLGQ
 HLGCLGSILSVVKKSTKFLPVLGWSMWFSEYLFERNWAKDKSTFKSHIERLE
 5 DFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKGFVSTVS
 HMRSFVPAVYDTTLTFPKTSPPTLLNLFAGQPIVLHIIKRRHAMKDIPESSDA
 VAQWCRDKFVEKDALLDKHNAEDAFSDQEFPIRSIKSLMVVISWVMVIFG
 ALKFLQWSSLLSSWKGIASFSGIGLGIVTLLMHILILSSQAERSTPAKVAQAKVK
 TELPSSTKVTNKG

10 [0182] SEQ ID NO: 68 CwLPAAT1

MAIPAAAVIFLFGILFFASGLIINLVQAVCFVLIWPLSKNTCRRINLVFQEMLLS
 ELLWLFHWRAKAEKLFTDPETYRLLGKEHALVMTNHRTDLDWMIGWVTG
 QHLGCLGSILSIKKSTKFLPVLGWSMWFSEYLFERNWAKDKSTFKSHIERL
 EDFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPHTKGFVSSV
 15 CHMRSFVPAVYDTTLTFPKNSPPPTLLNLFAGQPIVLHIIKRRHAMKDMPKSD
 DAVAQWCRDKFVKKDALLDKHNTEDTFSDQEFPIGRPIKSLMVVISWVVVIF
 GTLKFLQWSSLLSSWKGIASFSGIGLGIVTLLVHILILSSQAERSTPPKVAPAKLK
 TELSSTTKVINKGN

[0183] SEQ ID NO: 69 CwLPAAT2b

LGLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRLNRVVAELLWLELVWLIDW
 WAGVKIKVFTDHETFHLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGST
 LAVMKKSSKFLPVIGWSMWFSEYLFERSWAKDESTLKSGLNRLKDYPLPF
 WLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSF
 VPAIYDVTVAIPKTSPPTMLRMFKGQSSVDALLDKHNADDTFSGQELHDIGR
 25 PIKSLLVVISWAVLVVFGAVKFLQWSSLLSSWKGIASFSGIGLGIVTLLVHILILS
 SQAERSTSAKVAQAKVKTELSSSKKVKKNKG

[0184] SEQ ID NO: 70 CwLPAAT2a

LGLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRLNRVVAELLWLELVWLIDW
 WAGVKIKVFTDHETFHLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGST
 30 LAVMKKSSKFLPVIGWSMWFSEYLFERSWAKDESTLKSGLNRLKDYPLPF
 WLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLPRTKGFVSSVSHMRSF
 VPAIYDVTVAIPKTSPPTMLRMFKGQSSVLHVHLKRHLMKDLPESSDAVAQ
 WCRDIFVEKDVLLDKHNAEDTFSGQELQDIGRPVKSLLVISWTLLVIFGAVK
 FLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKEG
 35 ESSKMETDKEN

[0185] SEQ ID NO: 71 CgLPAAT1a

LAGWMGSSSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFERSWAKDEST
 LKSGLNRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASLGLPVPRNVLP
 RTKGFVSSVSHMRSFVPAIYDVTVAIPKTSPPTMIRMFKGQSSVLHVHLKRH
 40 VMKDLPESSDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLL
 VVISWAVLEVFGAVKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERS
 TPAKVAPAKPKNEGESSKAEMEKEK

[0186] SEQ ID NO: 72 CgLPAAT1b

LAGWMGSSSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDEST
 LKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASLGLPVPRNVLIP
 RTKGFVSSVSHMRSFVPAIYDVTVAIPKTSPPPTMIRMFKGQSSVLHVHLKRH
 VMKDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLL
 5 VRCFLVLSLIYLNGLMLKLRGPCLQVVISWAVLEVFGAVKFLQWSSLLSSWK
 GLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPAKPKNEGESSKAEMEKEK

[0187] SEQ ID NO: 73 CgLPAAT1c

LAGWMGSSSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDEST
 LKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASLGLPVPRNVLIP
 10 RTKGFVSSVSHMRSFVPAIYDVTVAIPKTSPPPTMIRMFKGQSSVLHVHLKRH
 VMKDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLL
 VVTSWAVLVISGAVKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSER
 STPAKVAPAKPKKEGESSKTEKDKEN

[0188] SEQ ID NO: 74 CpaLPAAT1

15 LGLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRINRVVAELLWLELVWLIDW
 WAGVKIKVFTDHEITLSLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGST
 LAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDENTLKSGNLRLKDYPLP
 WLALFVEGTRFTRAKLLAAQQYATSSGLPVPRNVLIPRTKGFVSSVSHMRSF
 VPAIYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPESDDAVAQ
 20 WCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLVIFGAVK
 FLQWSSLLSSWKGLAFSGVGLGIITLLMHILILFSQSERSTPAKVAPAKPKKDG
 ESSKTEIEKEN

[0189] SEQ ID NO: 75 CaLPAAT1

MAIAAAVIVPVSLFFVSGLIVNLVQAVCFVLIRPLFKNTYRRINRVVAELL
 25 WLELVWLIDWWAGVKIKVFTDHEITFHLMGKEHALVICNHKSDIDWLVGWV
 LAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKSG
 NLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPRTKGF
 VSSVSHMRSFVPAIYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHQMND
 LPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLIVISW
 30 AVLVVFGAVKFLQWSSLLSSWKGLAFSGIGLGVITLLMHILILFSQSERSTPAK
 VAPAKPKIEGESSKTEMEKEH

[0190] SEQ ID NO: 76 CaLPAAT3

MTIASAAVVFLLFGILLFTSGLIINLFQAFCSVLVWPLSKNAYRRINRVFAEFLPL
 EFLWLFHWWAGAKLKLFTDPETFRMLMGKEHALVIINHKEILDWVMVGWVLG
 35 QHLGCLGSILSVAKKSTKFLPVFGWSLWFSEYLFLEERNWAKDKKTLKSHIERL
 KDYPLPFWLIIFVEGTRFTRTKLLAAQQYAASAGLPVPRNVLIPHTKGFVSSVS
 HMRSFVPAIYDVTVAFPKTSPPPTMLKLFEGHFVELHVHIKRHAMKDLPESED
 AVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHHVGRPIKSLLVVISWVVVII
 FGALKFLQWSSLLSSWKGIAFSVIGLGTVALLMQILILSSQAERSIPAKETPANL
 40 KTELSSSKKVTNKEN

[0191] SEQ ID NO: 77 SalLPAAT1

MAIGAAIIVVPLGLLFMLSGLMVNLIQAICFILVRPLSKNMYRRVNRVVVELL
 WLELIWLIDWWGGVKVDVYADSETFQSLGKEHALVVSNHKSDIDWLVGWV
 LAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYVFLERSWAKDESTLKSGL

QRLKDFPRPFWLALFVEGTRFTQAKLLAAQEYAASTGLPIPRNVLPRTKGFV
SAVSNMRSFVPAIYDVTVAIPKTQPSPTMLRIFNRQPSVVHVRIKRHSMNQLP
PTDEGVAQWCKDIFVAKDALLDRHLAEGKFDEKEFKRIRRPISLLVISSWSF
LLFGVFKFLKWSALLSTWKGVAVSTAVLLLVTVVMYMFILFSQSERSSPRK
5 VAPSGPENG

[0192] SEQ ID NO: 78 CleptLPAAT1

MAIPAAVVFIFLFGLLFFSSGLIINLFQALCFVLIWPLSKNAYRRINRVFAELLS
ELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMG
QHFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWDKDKSTLKSHIERL
10 KDYPLPFWLVIFAEGTRFTRTKLLAAQQAASSGLPVPRNVLPRTKGFVSCV
NHMRSFVPAVYDLTVAFPKTSPPTLLNLFEGQSVVLHVHIKRHAMKDLPE
DDAVAQWCRDKFVEKDALLDKHNAEDTFSSQEVHHTGSRPIKSLLVVISWV
VVITFGALKFLQWSSWKGAFSVIGLGIVTLLMHMLILSSQAERSKPAKVTQA
KLKTELSISKKVTDKEN

15 [0193] SEQ ID NO: 79 ClopLPAAT1

MAIAAAVIFLFGLLFFASGLIINLFQALCFVLIWPLSKNAYRRINRVFAELLS
ELLCLFDWWAGAKLKLFTDPETLRLMGKEHALIINHMTELDWMVGWVMG
QHFGCLGSIIISVAKKSTKFLPVLGWSMWFSEYLYLERSWAKDKSTLKSHIERL
KDYPLPFWLVIFVEGTRFTRTKLLAAQEYAAASSGLPVPRNVLPRTKGFVSCV
20 NHMRSFVPAVYDVTVAFPKTSPQPTLLNLFEGRSIVLHVHIKRHAMKDLPE
SDAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHHTGRRPIKSLLVVM
SWVVTTFGALKFLQWSSWKGAFSVIGLGIVTLLMHVLILSSQAERSNPAKV
VQ AELNTELSISKKVTDKGN

[0194] SEQ ID NO: 80 CcrasLPAAT1a

MAIPAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLS
ELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMG
QHFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWDKDKSTLKSHIERL
KDYPLPFWLVIFAEGTRFTRTKLLAAQQAASSGLPVPRNVLPRTKGFVSSV
SHMRSFVPAIYDVTVAIPKTSPPTLIRMFKGQSSVLHVHLKRHVMDLPESD
30 DAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLE
VFGAVKFLQWSSLLSSWKGLAFSGIGLGITLLMHILILFSQSERSTPAKV
APA KAK

[0195] SEQ ID NO: 81 CcrasLPAAT1b

MAIPAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLS
35 ELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMG
QHFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWDKDKSTLKSHIERL
KDYPLPFWLVIFAEGTRFTRTKLLAAQQAASSGLPVPRNVLPRTKGFVSSV
SHMRSFVPAIYDVTVAIPKTSPPTLIRMFKGQSSVLHVHLKRHVMDLPESD
DAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVRCFLVLSLI
40 YLNGIILKLCGLCLQVVISWAVLEVFGAVKFLQWSSLLSSWKGLAFSGIGLGII
TLLMHILILFSQSERSTPAKVAKAK

[0196] SEQ ID NO: 82 CcrasLPAAT1c

MAIPAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLS
ELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMG

QHFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWDKDKSTLKSHIERL
 KDYPLPFWLVIFAEGTRFTRTKLLAAQQYAASSGLPVPRNVLPRTKGFVSSV
 SHMRSFVPAIYDVTVAIPKTSPPPTLIRMFKGQSSVLHVHLKRHVMKDLPESD
 DAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLE
 5 VFGAVKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPA
 KAKMEGESSKTEMEMEK

[0197] SEQ ID NO: 83 CcrasLPAAT1d

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLLS
 ELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGWVMG
 10 QHFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWDKDKSTLKSHIERL
 KDYPLPFWLVIFAEGTRFTRTKLLAAQQYAASSGLPVPRNVLPRTKGFVSSV
 SHMRSFVPAIYDVTVAIPKTSPPPTLIRMFKGQSSVLHVHLKRHVMKDLPESD
 DAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVRCFLVLSLI
 YLNGIILKLCGLCLQVVISWAVLEVFGAVKFLQWSSLLSSWKGLAFSGIGLGI
 15 TLLMHILILFSQSERSTPAKVAKAKMEGESSKTEMEMEK

[0198] SEQ ID NO: 84 CkoeLPAAT1

MAIAAAPVIFLFGLLFFASGLIINLFQAICFVLIWPLSKNAYRRINRVFAELLSE
 LLCLFDWWAGAKLKLFTDPETFRLMGKEHALVITNHKIDLDWMIGWILGQH
 FGCLGSVISIAKKSTKFLPIFGWSLWFSEYLFERNWAKDKRTLKSHIERMKD
 20 YPLPLWLILFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLPHTKGFVSSVSH
 MRSFVPAIYDVTVAIPKTSPPPTLIRMFKGQSSVLHVHLKRHLMKDLPESDDA
 VAQWCRDIFVEKDALLDKHNAEDTFSGQELQETGRPIKSLLVVISWAVLEVY
 GAVKFLQWSSLLSSWKGLAFSGIGLGLITLLMHILILFSQSERSTPAKVAPAKP
 KKEGESSKTEMEKEK

25 [0199] SEQ ID NO: 85 CkoeLPAAT2

MHVLLMVTFRFSSFFVFDNVQALCFVLIWPLSKSAYRKINRVFAELLSELL
 CLFDWWAGAKLKLFTDPETFRLMGKEHALVITNHKIDLDWMIGWILGQHFG
 CLGSVISIAKKSTKFLPIFGWSLWFSEYLFERNWAKDKRTLKSHIERMKDYP
 LPLWLILFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLPHTKGFVSSVSHMR
 30 SFVPAVYDVTVAFPKTSPPPTMLSLFEGQSVVLHVHIKRHAMKDLPSDDAV
 AQWCRDKFVEKDALLDKHNAEDTFSGQEVHHVGRPIKSLLVVISWMVVIIFG
 ALKFLQWSSLLSSWKGKAFAIGLGIATLLMHVLVVFSSQADRSNPAKVPPAK
 LNTELSSSKKVTNKEN

**35 EXAMPLE 5: EXPRESSION OF LPAATS TO IMPROVE SN-2
 SELECTIVITY IN *PROTOTHECA MORIFORMIS***

[0200] In the example we disclose genetically engineered *Prototheca moriformis*
 strains in which we have modified fatty acid and triacylglycerol biosynthesis to
 maximize the accumulation of Stearoyl-Oleoyl-Stearoyl (SOS) TAGs, and minimize
 the production of trisaturated TAGs. Oils from these strains resemble plant seed oils
 40 known as “structuring fats”, which have high proportions of Saturated-Oleate-
 Saturated TAGs and low levels of trisaturates. These structuring fats (often called
 “butters”) are generally solid at room temperature but melt sharply between 35-40°C.

[0201] Strains with high SOS and low trisaturates were obtained by three successive transformations, beginning with S5100, a classically improved derivative of S376 (improved to increase lipid titer), a wild type isolate of *Prototheca moriformis*. S5100 was transformed with a construct to which increased expression of *PmKASII-1* and
 5 ablated the *SAD2-1* allele. The resultant strain, S5780, produced oil with increased C18:0 and lower C16:0 content relative to S5100. S5780 was prepared according to the methods disclosed in co-owned application WO2013/158938 and as described below. C18:0 levels were increased further by transformation of S5780 with a construct overexpressing the C18:0-specific *FATA1* thioesterase gene from *Garcinia*
 10 *mangostana* (*GarmFATA1*), generating strain S6573. S6573 was disclosed in co-owned application WO2015/051319. Finally, accumulation of trisaturated TAGs was reduced by expression of genes encoding LPAATs from *Brassica napus*, *Theobroma cacao*, *Garcinia hombororiana* or *Garcinia indica* in S6573 as described below.

15 **Construct used for *SAD2* knockout and *PmKASII-1* overexpression in S5100 to produce S5780**

[0202] The sequence of the transforming DNA from the *SAD2-1* ablation, *PmKASII* over-expression construct, pSZ2624, is shown below. The construct is written as: pSZ2624: SAD2-1vD::PmKASII-1tp_PmKASII-1_FLAG-CvNR:CpACT-
 AtTHIC-CpEF1a::SAD2-1vE Relevant restriction sites are indicated in lowercase,
 20 bold, and are from 5'-3' PmeI, SpeI, AscI, ClaI, SacI, AvrII, EcoRV, AflII, KpnI, XbaI, MfeI, BamHI, BspQI and PmeI. Underlined sequences at the 5' and 3' flanks of the construct represent genomic DNA from *P. moriformis* that enable targeted integration of the transforming DNA via homologous recombination at the *SAD2-1* locus. The *SAD2-1* 5' integration flank contained the endogenous *SAD2-1* promoter,
 25 enabling the *in situ* activation of the *PmKASII* gene. Proceeding in the 5' to 3' direction, the region encoding the *PmKASII* plastid targeting sequence is indicated by lowercase, underlined italics. The sequence that encodes the mature *PmKASII* polypeptide is indicated with lowercase italics, while a 3xFLAG epitope encoding sequence is in bold italics. The initiator ATG and terminator TGA for *PmKASII*-
 30 *FLAG* are indicated by uppercase italics. The 3' UTR of the *Chlorella vulgaris* nitrate reductase (*CvNR*) gene is indicated by small capitals. Two spacer regions are represented by lowercase text. The *CpACT* promoter driving the expression of the *AtTHIC* gene (encoding 4-amino-5-hydroxymethyl-2-methylpyrimidine synthase

activity, thereby permitting the strain to grow in the absence of exogenous thiamine) is indicated by lowercase, boxed text. The initiator ATG and terminator TGA for *AtTHIC* are indicated by uppercase italics, while the coding region is indicated with lowercase italics. The 3' UTR of the *Chlorella protothecoides EF1a* (*CpEF1a*) gene is indicated by small capitals. The use of *THIC* as a selection marker was described in co-owned applications WO2011/150410 and WO2013/150411.

[0203] SEQ ID NO: 86 pSZ2624 Nucleotide sequence of the transforming DNA

[illegible]

TTTATCAAACAGCCTCAGTGTGTTTGATCTTGTGTGTACGCGCTTTTTCGA
 GTTGCTAGCTGCTTGTGCTATTTGCGAATACCACCCCCAGCATCCCCTTCC
 CTCGTTTCATATCGCTTGCATCCCAACCGCAACTTATCTACGCTGTCCTGCT
 ATCCCTCAGCGCTGCTCCTGCTCCTGCTCACTGCCCCCTCGCACAGCCTTGG
 5 TTTGGGCTCCGCCTGTATTCTCCTGGTACTGCAACCTGTAAACCAGCACTG
 CAATGCTGATGCACGGGAAGTAGTGGGATGGGAACACAAATGGAAAGCT
 TAATTA**Agagctc**gcgctcgaacagagcgcgcagaggaacgctgaaggctcgcctctgtcgacctcagcgc
 ggcatacaccacaataaccacctgacgaatgcgcttggttcttcgtccattagcgaagcgtccggttcacacacgtgccacg
 ttggcgaggtggcaggtgacaatgatcgggtggagctgatggtcgaaacgttcacag**cctaggtgatatccatcttaagga**
 10 tctaagtaagattcgaagcgtcgacctgcccggacggactgcagccccatgtcgtagtaccgccaatgtaagtgggct
 ggcggttccctgtacgtgagtcaacgtcactgcacgcgcaccacctctcgaccggcaggaccaggcatcgcgagataca
 gcgcgagccagacacggagtgcgagctatgcgcacgtcccaact**ggtagc**agtttaggtccagcgtccgtggggggg
 ggacgggctgggagcttgggcccgggaagggaagacgatgcagtcctctggggagtcacagccgactgtgtgtgtg
 cactgtgcggccccgcagcactcacacgcaaatgcctggcgacaggcaggccctgtccagtgcacatccacggtccc
 15 tctcatcaggctcacctgtcattgacataacggaatgcgtaccgctctttcagatctgtccatccagagaggggagcagg
 ctccccaccgacgtgtcaactgtctcctgcccaaccgaaaacattattgtttgagggggggggggggggggcagatt
 gcatggcgggatatctcgtgaggaacatcactgggacactgtggaacacagtgaagtgcagtatgcagagcatgtatgcta
 ggggtcagcgcaggaagggggcctttccagctcctccatgccactgcaccgtatccacgactcaccaggaccagcttctt
 gatcggcttccgctcccgtggacaccagtgtgtagcctctggactccaggtatgcgtgcaccgcaaaggccagccgatcg
 20 tgccgattcctgggtggagatatgagtcagcaacttggggctcagagtgcacactggggcacgatacgaacaacatc
 tacaccgtgtcctccatgctgacacaccacagcttcgctccacctgaatgtgggcgcatgggcccgaatcacagccaatgt
 cgctgctgccataatgtgatccagacctctccgccagatgccgagcggatcgtgggcgctgaatagattcctgtttcgt
 cactgtttgggtcctttcttctcgtcgtcgatgcgcgtctcgaaacaggctgcgtcgggctttcggatccctttgtcctccg
 tcaccatctgcgcgcccgaagtgtgaccctgggctgataccaggggttgagggtattaccgctcaggccattccc
 25 agcccggttcaattcaaagtctggggccaccacctccgcccgtctgtctgatcactccacattcgtgcatacactacgttca
 agtctgatccaggcgtgtctcgggacaagggtgtgcttgagttgaatctcaaggaccactccagcacagctgctggttga
 ccccgccctcgcaat**ctaga**ATGccgcgtccgtccactgcacctgatgtccgtgggtcgaacaacaagaaccac
 tccgcccgcaccaagctgcccaactcctccctgctgcccggcttcgacgtgggtgggtccaggccgcggccacccgcttca
 agaaggagacgacgaccacccgcgccacgctgacgttcgaccccccaagaccaactccgagcgcgccaagcag
 30 cgcaagcacaccategacccctcctccccgacttccagccccctccttcgaggagtgttccccaagttccacga
 aggagcacaaggaggtgggtgcacgaggagtcgggccacgtcctgaagggtcccttccgcgcgtgcacctgtccgg
 cggcgagccccgcttcgacaactacgacacgtcggccccccagaacgtcaacgccacatcggccttggegaagctg
 cgcaaggagtggatcgaccgccgcgagaagctgggcacgccccgtacacgcagatgtaclacgcgaagcagggc
 atcalcacggaggagatgctgactgcgcgacgcgcgagaagctggacccccagttcgtccgctccgaggtcgcgcg
 35 gggcgcgcccacatccccccaacaagaagcacctggagctggagcccatgacgtgggcccgaagttcctgggtga
 aggtgaacgcgaacatcggaactccgcgtggcctcctccatcgaggaggaggtctacaaggtgcagttgggcccac
 catgtggggcgcgcacacatcatggacctgtccacgggcgccacatccacgagacgcgcgagttggatcttgcgc
 aactccgcggtccccgtgggcacgtccccatctaccaggcgtcggagaaggtggacggcaltcgcggagaacctga
 actgggaggtgttccgcgagacgctgatcgagcaggccgagcagggcgtggactacttcacgatccacgcgggcgt
 40 gctgtcgcgtacatccccctgaccgccaagcgcctgacgggcacgtgtcctcgcggcggtccatccacgcgaagtg
 gtgcttggcctaccacaaggagaacttcgctacgagcactgggacgacatcctggacatctgaaccagttacgacg
 tgcctgtccatcgggcagggcctgcgcgcccggtccatctacgacgccaacgacacggccccagttcgcgagctgc
 tgaccagggcgagctgacgcgcgcgtgggagaaggacgtgcaggtgatgaacgagggccccggccacgtg
 cccatgcacaagatccccgagaacatgcagaagcagctggagtggtgcaacgagggcgccttctacacctggggcc
 45 ccttgacgacccgacatcgccccggctacgaccacatcacctccgccatcggcgcggcccaacatcggcgccttggg
 caccgcccgtgtgtgtacgtgacgccaaggagcactgggcccgtcccaaccgcgacgacgtgaagggcggggcgtc
 atgcctacaagatcgccgccacgcggcgacctggccaagcagacccccacgcccaggcgtgggacgacgcg
 ctgtccaagggcgcgttcgagttccgtcgtggatggaccagttcgcgtgtccctggaccccatgacggcgatgtccttca
 cgacgagacgctgcccgcggacggcggaagggtgcgccacttctgtccatgtcgggcccccaagttctgtccatgaa

gatcacggaggacatccgcaagtagccgaggagaaacggctacggctccgccgaggaggccatccgccaggccat
 ggacgccatgtccgaggagtcaacatcgccaagaagacgatctccggcgagcagcacggcgaggctggcgggcga
 gatctacctgcccagtgctcaagggcgcgagaagTGA**caattg**ACGGAGCGTCGTGCGGG
 AGGGAGTGTGCCGAGCGGGGAGTCCCGGTCTGTGCGAGGCCCGGCAGCTG
 5 ACGCTGGCGAGCCGTACGCCCCGAGGGTCCCCCTCCCCTGCACCCTCTTCC
 CCTTCCCTCTGACGGCCGCGCCTGTTCTTGCATGTTACGCGAC**ggatcc**TAGG
 GAGCGACGAGTGTGCGTGCGGGGCTGGCGGGAGTGGGACGCCCTCCTCGC
 TCCTCTCTGTTCTGAACGGAACAATCGGCCACCCCGCGCTACGCGCCACG
 CATCGAGCAACGAAGAAAACCCCCGATGATAGGTTGCGGTGGCTGCCGG
 10 GATATAGATCCGGCCGCACATCAAAGGGCCCCTCCGCCAGAGAAGAAGCT
 CCTTCCCAGCAGACTCCTTCTGCTGCCAAAACACTTCTCTGTCCACAGCA
 ACACCAAAGGATGAACAGATCAACTTGCGTCTCCGCGTAGCTTCCTCGGC
 TAGCGTGCTTGCAACAGGTCCCTGCACTATTATCTTCCTGCTTTCCTCTGA
 ATTATGCGGCAGGCGAGCGCTCGCTCTGGCGAGCGCTCCTTCGCGCCGCC
 15 CTCGCTGATCGAGTGTACAGTCAATGAATGGTCCTGGGCGAAGAACGAGG
 GAATTTGTGGGTAAAACAAGCATCGTCTCTCAGGCCCCCGGCGCAGTGGCC
 GTTAAAGTCCAAGACCGTGACCAGGCAGCGCAGCGCGTCCGTGTGCGGGC
 CCTGCCTGGCGGCTCGGCGTGCCAGGCTCGAGAGCAGCTCCCTCAGGTCG
 CCTTGGACGGCCTCTGCGAGGGCCGGTGAGGGCCTGCAGGAGCGCCTCGAG
 20 CGTGGCAGTGGCGGTTCGTATCCGGGTGCGCGGTACCGCCTGCGACTCGC
 CATCCgaagagcg**tttaaac**

[0204] Construct D1683 (pSZ2624), was transformed into S5100. Primary
 transformants were clonally purified and grown under standard lipid production
 conditions at pH 5. Integration of pSZ2624 at the *SAD2-1* locus was verified by DNA
 25 blot analysis. The fatty acid profiles and lipid titers of lead strains were assayed in 50-
 mL shake flasks (Table 8). Simultaneous ablation of *SAD2-1* and over-expression of
PmKASII (driven *in situ* by the *SAD2-1* promoter) resulted in C18:0 levels up to
 26.1%. C16:0 accumulation was reduced from 15.3% in S5100 to $\leq 6\%$ the strains
 derived from D1683, demonstrating that *PmKASII-1* over-expression promoted the
 30 elongation of C16:0 to C18:0. S5780 was chosen for further development as it had the
 highest lipid titer relative to the S5100 parent.

[0205] Table 8. Fatty acid profiles of *SAD2-1* ablation, *PmKASII-1* overexpression
 strains derived from D1683-1, compared to the S5100 parent.

Primary		S5100;T531;D1683.1					
Strain		S5100	S5780	S5781	S5782	S5783	S5784
Fatty Acid Area %	C14:0	0.7	0.7	0.8	0.7	0.7	0.7
	C16:0	15.3	5.9	6.0	6.0	5.8	5.8
	C16:1	0.5	0.1	0.0	0.1	0.0	0.0
	C18:0	4.0	25.6	26.1	26.0	25.0	25.3
	C18:1	71.0	55.7	54.5	54.6	56.3	55.6

	C18:2	7.3	8.0	8.5	8.5	8.1	8.4
	C18:3 α	0.5	0.7	0.8	0.8	0.7	0.7
	C20:0	0.3	1.8	1.9	1.8	1.8	1.8
	C20:1	0.2	0.6	0.6	0.6	0.7	0.7
	C22:0	0.1	0.2	0.3	0.3	0.3	0.2
	C24:0	0.1	0.4	0.4	0.4	0.4	0.4
	saturates	20.6	34.7	35.6	35.4	34.1	34.5

[0206] We disclose additional methods of elevating C18:0 levels that can be used in conjunction with *SAD2* knockout and *KASII* over-expression. Previously we described acyl-ACP thioesterases from *Brassica napus* (*BnFATA*) (Co-owned application WO2012/106560), *Garcinia mangostana* (*GarmFATA1*) (Co-owned application WO2015/051319) and *Theobroma cacao* (*TcFATA*) (Co-owned application WO2013/158938) with specificity towards cleavage of C18:0-ACP, and we observed that average C18:0 levels were higher in strains in which we replaced the native BnFATA transit peptide with the *Chlorella protothecoides* SAD1 transit peptide (CpSAD1tp). A DNA construct was made for expression of a chimeric gene encoding CpSAD1tp fused to the predicted GarmFATA1 mature polypeptide and a FLAG tag sequence.

[0207] The sequence of the transforming DNA from the *GarmFATA1* expression construct pSZ3204 is shown below. The construct is written as pSZ3204:
6SA::CrTUB2-ScSUC2-CvNR:PmSAD2-2-CpSAD1tp_GarmFATA1_FLAG-CvNR::6SB. Relevant restriction sites are indicated in lowercase, bold, and are from 5'-3' BspQI, KpnI, XbaI, MfeI, BamHI, AvrII, EcoRV, SpeI, AscI, ClaI, AflII, SacI and BspQI. Underlined sequences at the 5' and 3' flanks of the construct represent genomic DNA from *P. moriformis* that enable targeted integration of the transforming DNA via homologous recombination at the 6S locus. Proceeding in the 5' to 3' direction, the *CrTUB2* promoter driving the expression of *Saccharomyces cerevisiae* *SUC2* (*ScSUC2*) gene, enabling strains to utilize exogenous sucrose, is indicated by lowercase, boxed text. The initiator ATG and terminator TGA of *ScSUC2* are indicated by uppercase italics, while the coding region is represented by lowercase italics. The 3' UTR of the *CvNR* gene is indicated by small capitals. A spacer region is represented by lowercase text. The *P. moriformis* *SAD2-2* (*PmSAD2-2*) promoter driving the expression of the chimeric *CpSAD1tp_GarmFATA1_FLAG* gene is indicated by lowercase, boxed text. The initiator ATG and terminator TGA are

indicated by uppercase italics; the sequence encoding CpSAD1tp is represented by lowercase, underlined italics; the sequence encoding the GarmFATA1 mature polypeptide is indicated by lowercase italics; and the 3X FLAG epitope tag is represented by uppercase, bold italics. A second *CvNR* 3' UTR is indicated by small capitals.

[0208] SEQ ID NO: 87 pSZ3204

gctcttcGCCGCCGCCACTCCTGCTCGAGCGCGCCCCGCGCGTGCGCCGCCAGC
GCCTTGGCCTTTTCGCCGCGCTCGTGCGCGTCGCTGATGTCCATCACCAGG
TCCATGAGGTCTGCCTTGCGCCGGCTGAGCCACTGCTTCGTCCGGGCGGCC
10 AAGAGGAGCATGAGGGAGGACTCCTGGTCCAGGGTCCTGACGTGGTCGCG
GCTCTGGGAGCGGGCCAGCATCATCTGGCTCTGCCGCACCGAGGCCGCCT
CCAACTGGTCCTCCAGCAGCCGCAGTCGCCGCCGACCCTGGCAGAGGAAG
ACAGGTGAGGGGGGTATGAATTGTACAGAACAACCACGAGCCTTGTCTAG
GCAGAATCCCTACCAGTCATGGCTTTACCTGGATGACGGCCTGCGAACAG
15 CTGTCCAGCGACCCTCGCTGCCGCCGCTTCTCCCGCACGCTTCTTTCCAGC
ACCGTGATGGCGCGAGCCAGCGCCGCACGCTGGCGCTGCGCTTCGCCGAT
CTGAGGACAGTCGGGGAACCTCTGATCAGTCTAAACCCCTTGCGCGTTAG
TGTTGCCATCCTTTGCAGACCGGTGAGAGCCGACTTGTTGTGCGCCACCCC
CCACACCACCTCCTCCCAGACCAATTCTGTACACCTTTTTGGCGAAGGCATC
20 GGCCTCGGCCTGCAGAGAGGACAGCAGTGCCCAGCCGCTGGGGGTTGGC
GGATGCACGCTCAggtaccctttcttgcgctatgacacttcagaaaaggtagggcggtgcgagacggct
tcccggcgctgcatgcaacaccgatgatgcttcgacccccgaagctccttcggggctgcatggcgctccgatgccgct
ccagggcgagcgctgtttaatagccaggccccgattgcaaagacattatagcgagctaccaaagccatattcaaacacc
tagatcactaccacttctacacaggccactcgagcttgatcgcaactccgctaagggggcgctcttctcttcttctcagtc
25 acaaccgcgaaactctagaatatcaATGctgctgcaggccttctgttctgctggccggcttcgccgcaagatcagc
gctccatgacgaacgagacgtccgacggccccctgggtgcacttcacccccacaagggtggatgaacgacccca
acggcctgtggtacgacgagaaggacgccaagtggcacctgtacttcagtlacaacccgaacgacacccgtctgggg
gacgccctgttctggggccacgccaagtcgacgacctgaccaactgggaggaccagcccategccategccccga
agegcaacgactccggcgcccttctccggctccatgggtgggtggaactacaacaacacctccggcttcttcaacgacaccat
30 cgacccgcgccagcgctgcgtggccatctggacctacaacaccccgagtcgaggagcagtlacatctctacagcc
tggacggcggttacaccttcaccgagtlaccagaagaaccccgctgctggccgccaactccacccagtlcccgacccg
aaggcttctgtgtacgagccctccagaagtggatcatgaccgcggccaagtcccaggactacaagatcgagatctac

tcctccgacgacctgaagtcctggaagctggagtcgcggttcgccaacgagggcttcctcggctaccagtlacgagtg
 cccggcctgategaggccccaccgagcaggaccccgcaagtcctactgggtgatgttcatctccatcaacccggc
 gccccggcggcggtccttcaaccagtlacttcgctggcagcttcaacggcaccacttcgaggecttcgacaaccagt
 cccgcgtgggtggacttcggcaaggactactacgccccgcagaccttcttcaacaccgacccgacclacgggagcgccc
 5 tgggcategcgtgggcctccaactgggagtlactccgcttcgtgccaccaacccctggcgctcctccatgtccctcgtg
 cgcaagttctccctcaacaccgagtlaccaggccaacccggagacggagctgatcaacctgaaggccgagccgatcc
 tgaacatcagcaacgccccctggagccggttcgccaccaacaccacgttgacgaaggccaacagctacaacgt
 cgacctgtccaacagcaccggcaccctggagttcgagctgggtgtacgcggtcaacaccacccagacgatctccaagt
 ccgtgttcgaggacctctccctctgggtcaaggcgctggaggaccccgaggagtlacctccgcatgggcttcgaggtgtc
 10 cgcgtcctccttcttcctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaaccaaccgcat
 gagegtgaacaaccagccccctcaagagcgagaacgacctgtcctactacaagggtgtacggcttgcgtggaccagaaca
 tcttggagctgtacttcaacgaecggcgacgtcgtgtccaccaacacctacttcatgaccacgggaacgccttgggctc
 cgtgaacatgacgacgggggtggacaacctgttclacatcgacaagttccagggtgcgcgaggtcaagTGA**caattg**
 GCAGCAGCAGCTCGGATAGTATCGACACACTCTGGACGCTGGTCGTGTGA
 15 TGGACTGTTGCCGCCACACTTGCTGCCTTGACCTGTGAATATCCCTGCCGC
 TTTTATCAAACAGCCTCAGTGTGTTTGATCTTGTGTGTACGCGCTTTTGCG
 AGTTGCTAGCTGCTTGTGCTATTTGCGAATACCACCCCCAGCATCCCCTTC
 CCTCGTTTCATATCGCTTGCATCCCAACCGCAACTTATCTACGCTGTCCTG
 CTATCCCTCAGCGCTGCTCCTGCTCCTGCTCACTGCCCCCTCGCACAGCCTT
 20 GGTTTGGGCTCCGCCTGTATTCTCCTGGTACTGCAACCTGTAAACCAGCAC
 TGCAATGCTGATGCACGGGAAGTAGTGGGATGGGAACACAAATGGAG**ggatc**
 ccgcgtctgaacagagcgcgagaggaaacgtgaaggctcgcctctgtcgacctcagcgcgccatacaccacaata
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cgacaacctttctgctgtcaccacgacccacgatgcaacgcgacacgacccgggtgggactgatcgggttcactgcacctgc
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5 gccagattggtgtccgataccttgatttgccatcagcgaaacaagacttcagcagcgagcgtatttggcgggctgctacca
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ctcccgccgcgcgcaggatagactctagtccaaccaatcgaca**actagtATG****gccaccgcatccactttctcggcgtt**
10 **caatgcccgtcgcggcgacctgcgtcgtcggcgggctccgggccccggcgcccagcgaggccccctcccgtgcgc**
ggcgcgccatcccccccgcatcatcgtgggtgtcctcctcctcctccaagggtgaacccccgaagaccgaggccgtg
gtgtcctcggcctggccgaccgcctgcgcctgggtcctcctgaccgaggacggcctgtcctacaaggagaagtgcac
gtgcgtgtctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgccaacctgtgcaggaggtgggt
gcaaccacgcccagtcctgggtactccaccggcggtttcaccacccccaccatgcgcaagctgcgcctgatct
15 **gggtgaccgcccgcaltgcacatcgagatctacaagttaccccgctgggtccgacgtgggtggagatcgagtcctggggc**
cagggcgagggcaagatcggcacccgcccgcgactggatcctgcgcgactacgccaccggccagggtgatcgccgc
gccacctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtggacgtgcgcgacgagt
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tggaggaccccccccagttactccaagctgggcctgggtccccgcccgcgcgacctggacatgaaccagcaactgaac
20 **aacgtgacctacatcggtgtgggtgtcgtggagttcatgccccaggagatcatcgacacccacgagctgcagaccatcac**
cctggactaccgcccgcgagtgccagcagcagcagcgtgggtggactccctgacctccccgagccctccgaggacgcc
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gcacctgtcgcgcctgtccggcaacggcctggagatcaaccgcccgcgacccagtggtggcgaagaagccccccc
cATGGACTACAAGGACCACGACGGCGACTACAAGGACCACGACATCGACTA
25 **CAAGGACGACGACGACAAGTGA****atcgatagatctcttaagGCAGCAGCAGCTCGGAT**
AGTATCGACACACTCTGGACGCTGGTCTGTGTGATGGACTGTTGCCGCCAC
ACTTGCTGCCTTGACCTGTGAATATCCCTGCCGCTTTTATCAAACAGCCTC
AGTGTGTTTGATCTTGTGTGTACGCGCTTTTGCGAGTTGCTAGCTGCTTGT
GCTATTTGCGAATACCACCCCCAGCATCCCCCTTCCCTCGTTTCATATCGCT
30 **TGCATCCCAACCGCAACTTATCTACGCTGTCCTGCTATCCCTCAGCGCTGC**
TCCTGCTCCTGCTCACTGCCCCCTCGCACAGCCTTGGTTTGGGCTCCGCCTG
TATTCTCCTGGTACTGCAACCTGTAAACCAGCACTGCAATGCTGATGCACG
GGAAGTAGTGGGATGGGAACACAAATGGA**aagcttaattaagagctcTTGTTTTCCA**

GAAGGAGTTGCTCCTTGAGCCTTTCATTCTCAGCCTCGATAACCTCCAAAG
 CCGCTCTAATTGTGGAGGGGGTTCGAATTTAAAAGCTTGGAATGTTGGTTC
 GTGCGTCTGGAACAAGCCCAGACTTGTTGCTCACTGGGAAAAGGACCATC
 AGCTCCAAAAAACTTGCCGCTCAAACCGCGTACCTCTGCTTTCGCGCAATC
 5 TGCCCTGTTGAAATCGCCACCACATTCATATTGTGACGCTTGAGCAGTCTG
 TAATTGCCTCAGAATGTGGAATCATCTGCCCCCTGTGCGAGCCCATGCCAG
 GCATGTCGCGGGGCGAGGACACCCGCCACTCGTACAGCAGACCATTATGCT
 ACCTCACAAATAGTTCATAACAGTGACCATATTTCTCGAAGCTCCCCAACGA
 GCACCTCCATGCTCTGAGTGGCCACCCCCCGGCCCTGGTGCTTGCGGAGG
 10 GCAGGTCAACCGGCATGGGGCTACCGAAATCCCCGACCGGATCCCACCAC
 CCCC GCGATGGGAAGAATCTCTCCCCGGGATGTGGGCCCACCACCAGCAC
 AACCTGCTGGCCCAGGCGAGCGTCAAACCATAACCACACAAATATCCTTGG
 CATCGGCCCTGAATTCCTTCTGCCGCTCTGCTACCCGGTGCTTCTGTCCGA
 AGCAGGGGTTGCTAGGGATCGCTCCGAGTCCGCAAACCCTTGTCGCGTGG
 15 CGGGGCTTGTTTCGAGCTTgaagagc

[0209] Construct D1940 (pSZ3204), was transformed into the S5780 parent strain. Primary transformants were clonally purified and grown under standard lipid production conditions at pH 5. Integration of pSZ3204 at the *6S* locus was verified by DNA blot analysis. The fatty acid profiles and lipid titers of lead strains were assayed in 50-mL shake flasks (Table 9). Over-expression of *GarmFATA1* (driven by the *SAD2-2* promoter) resulted in C18:0 levels up to 54.3%. C16:0 levels were comparable in strains derived from D1940 and the S5780 parent. S6573 was chosen for further development as it had the highest lipid titer of the strains with >50% C18:0.

[0210] Table 9. Fatty acid profiles of *GarmFATA1* overexpressing stable strains derived from D1940 primary transformants.

Primary			D1683.1	D1940.19			D1940.20	D1940.23	D1940.46	D1940.5
Strain		S5100	S5780	S6571	S6572	S6573	S6574	S6575	S6578	S6580
Fatty Acid Area %	C14:0	0.7	0.0	0.8	0.0	0.8	0.7	0.7	0.0	0.0
	C16:0	18.0	5.9	6.3	6.6	6.3	5.0	5.1	5.0	5.3
	C16:1	0.5	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1
	C18:0	3.9	29.0	52.7	54.3	53.7	43.1	46.0	45.4	47.9

C18:1	69.8	54.3	31.4	30.1	30.5	41.5	38.5	40.0	37.2
C18:2	5.9	6.4	5.7	5.8	5.6	6.3	6.2	6.1	6.2
C18:3 α	0.5	0.7	0.6	0.6	0.6	0.6	0.5	0.6	0.5
C20:0	0.3	2.4	1.8	1.6	1.7	2.1	2.0	2.0	2.0
C20:1	0.1	0.6	0.1	0.1	0.1	0.2	0.1	0.1	0.1
C22:0	0.1	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.2
C24:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
saturates	23.1	37.7	61.9	62.8	62.8	51.2	54.2	52.7	55.5

[0211] Lysophosphatidic acid acetyltransferase (LPAAT) enzymes are responsible for the transfer of acyl groups to the *sn*-2 position on the glycerol backbone. We disclose here that we can reduce the accumulation of excessive amounts of trisaturates in our high SOS strains by expressing heterologous *LPAAT* genes which were better than the endogenous acyltransferases at discriminating against saturated fatty acids. Expression of *LPAT2* homologs from *B. napus*, *T. cacao*, *Garcinia hombroriana* and *Garcinia indica* and their effect on the formation of trisaturated TAGs in the high-C18:0 S6573 strain is disclosed below.

10 [0212] The sequence of the transforming DNA from the *BnLPAT2(Bn1.13)* expression construct pSZ4198 is shown below. The construct is written as pSZ4198: PLOOP::PmHXT1-ScarMEL1-CvNR::PmSAD2-2v2-BnLPAT2(Bn1.13)-CvNR::PLOOP. Relevant restriction sites are indicated in lowercase, bold, and are from 5'-3' BspQI, KpnI, SpeI, SnaBI, EcoRI, SpeI, ClaI, BglII, AflII, HindIII, SacI and BspQI. Underlined sequences at the 5' and 3' flanks of the construct represent genomic DNA from *P. moriformis* that enable targeted integration of the transforming DNA via homologous recombination at the *PLOOP* locus. Proceeding in the 5' to 3' direction, the *PmHXT1* promoter driving the expression of *S. carlbergensis MEL1* (*ScarMEL1*) gene, enabling strains to utilize exogenous melibiose, is indicated by lowercase, boxed text. The initiator ATG and terminator TGA of *ScarMEL1* are indicated by uppercase italics, while the coding region is represented by lowercase italics. The 3' UTR of the *CvNR* gene is indicated by small capitals. The *P. moriformis SAD2-2v2* promoter driving the expression of the *BnLPAT2(Bn1.13)* gene is indicated by lowercase, boxed text. The initiator ATG and terminator TGA are indicated by uppercase italics; the sequence encoding *BnLPAT2(Bn1.13)* is represented by lowercase, underlined italics. A second *CvNR* 3' UTR is indicated by

small capitals. The *Brassica napus* LPAAT2(BN1.13) sequence is from Genbank accession GU045434.

[0213] SEQ ID NO: 88: Nucleotide sequence of the transforming DNA from pSZ4198

5 gctcttcgctAACGGAGGTCTGTCACCAAATGGACCCCGTCTATTGCGGGAAA
CCACGGCGATGGCACGTTTCAAACTTGATGAAATACAATATTCAGTATG
TCGCGGGCGGGCGACGGCGGGGAGCTGATGTCGCGCTGGGTATTGCTTAAT
CGCCAGCTTCGCCCCCGTCTTGGCGCGAGGCGTGAACAAGCCGACCGATG
TGCACGAGCAAATCCTGACACTAGAAGGGCTGACTCGCCCGGCACGGCTG
10 AATTACACAGGCTTGCAAAAATACCAGAATTTGCACGCACCGTATTCGCG
GTATTTTGTGGACAGTGAATAGCGATGCGGCAATGGCTTGTGGCGTTAG
AAGGTGCGACGAAGGTGGTGCCACCACTGTGCCAGCCAGTCCTGGCGGCT
CCCAGGGCCCCGATCAAGAGCCAGGACATCCAACTACCCACAGCATCAA
CGCCCCGGCCTATACTCGAACCCCACTTGCACTCTGCAATGGTATGGGAA
15 CCACGGGGCAGTCTTGTGTGGGTGCGGCCTATCGCGGTGCGCGAAGACCG
GGA**Aggtacc**gcggtgagaatcgaaaatgcatcgtttctaggttcggagacggtcaattccctgctcggcggaatctgt
cgggtcaagctggccagtggacaatgttgctatggcagcccgcgacatgggctcccgcgcccacatcaggagccca
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20 cctcggaacgtctcgtagggcaacgccccgagtcggcgaggccgtaaacattgtttctgggtgtcggagtgggca
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25 cccattctgtgccacacgccgacgaggaccaatccccggcatcagccttcacgaggtgctgcgcgacatataagccg
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30 ggcatgggccaactgctccgaccactgcacaacaactccttctgttccgcatgtactctccgaggcgagttacagt
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gaagtacgacaactgtctacaacaaggccaggttcggcaacgcccagatctctaccaccgctacaaggccatgtccg
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35 acggcgacgagtagcactgcaagtagcgcgggttcactgtcctcatgaacatctgaacaaggccgccccatg
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40 acggcgaccagggtcgtggcgctgtgaacggcggtccgtgtccgccccatgaacacgacctggaggagatcttct
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45 AGCTCGGATAGTATCGACACACTCTGGACGCTGGTCGTGTGATGGACTGT
TGCCGCCACACTTGCTGCCTTGACCTGTGAATATCCCTGCCGCTTTTATCA
AACAGCCTCAGTGTGTTTGATCTTGTGTGTACGCGCTTTTGCAGTTGCTA

GCTGCTTGTGCTATTTGCGAATACCACCCCCAGCATCCCCTTCCCTCGTTT
 CATATCGCTTGCATCCCAACCGCAACTTATCTACGCTGTCCTGCTATCCCT
 CAGCGCTGCTCCTGCTCCTGCTCACTGCCCCCTCGCACAGCCTTGTTTTGGG
 CTCCGCCTGTATTCTCCTGGTACTGCAACCTGTAAACCAGCACTGCAATGC
 5 TGATGCACGGGAAGTAGTGGGATGGGAACACAAATGGAAagctgtagaattcctgg
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 ccggtgggactgatcggtcactgcacctgcatgcaattgtcacaagcgcatactccaatcgatccgtttgatttctgtgaaa
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 caaatcgaggcgatccggagattgggatctgatccgagcttgaccagatccccacgatgcggcacgggaactgcat
 10 cgactcggcgcggaaccagctttcgtaaattgccagattgggtgctcgataccttgatttgccatcagcgaaacaagacttca
 gcagcgagcgtatttggcgggctgctaccaggggtgcatacattgccatttctgtctggaccgctttaccggcgagagg
 gtgagttgatggggtggcaggcatcgaaacgcgcgtgcatggtgtgtgtgtgtgttctggctgcacaatttcaatagtcgg
 atgggacgaggtagaattgggtgttcgctcgcgtgcatgcctcgccccgtcggtgtcatgaccgggactggaatccc
 cctcgagacctcctgtaacgctcccgactctcccgcccgcgcgagcatagactctagttaaccaatcgacaactag**t**
 15 **ATG**gccatggcgcgcgctgctgctgccccgggcatcctgttctcatcctcgccctgggtgggtaacctgtgcagg
 ccatctgtactgtgctgatccgccccctgtccaagaacactaccgcaagatcaaccgcgtgggtggcgagacctgt
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 25 tccgacgacgccatcgcccagtggtgccgcgaccagttcgtggccaaggacgcccctgtggacaagcacatcgccgc
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 ggctgggcatcatcaccctgtgcatgcagatcctgatccgctcctccagtcgagcgctccacccccgccaagggtgg
 tggcgccaagcccaaggacaaccacaacgactccggctcctcctccagaccgaggtggagaagcagaag**TGA**
 30 **atcgatagatctcttaag**GCAGCAGCAGCTCGGATAGTATCGACACACTCTGGACG
 CTGGTTCGTGTGATGGACTGTTGCCGCCACACTTGCTGCCTTGACCTGTGAA
 TATCCCTGCCGCTTTTATCAAACAGCCTCAGTGTGTTTGATCTTGTGTGTAC
 GCGCTTTTTCGAGTTGCTAGCTGCTTGTGCTATTTGCGAATACCACCCCCA
 GCATCCCCTTCCCTCGTTTCATATCGCTTGCATCCCAACCGCAACTTATCT
 35 ACGCTGTCCTGCTATCCCTCAGCGCTGCTCCTGCTCCTGCTCACTGCCCCCT
 CGCACAGCCTTGTTTTGGGCTCCGCCTGTATTCTCCTGGTACTGCAACCTG
 TAAACCAGCACTGCAATGCTGATGCACGGGAAGTAGTGGGATGGGAACA
 CAAATGGAA**agcttaattaagagetc**AGCGGCGACGGTCTGCTACCGTACGACGTT
 GGGCACGCCCATGAAAGTTTGTATACCGAGCTTGTTGAGCGAACTGCAAG
 40 CGCGGCTCAAGGATACTTGAACTCCTGGATTGATATCGGTCCAATAATGG
ATGGAAAATCCGAACCTCGTGCAAGAACTGAGCAAACCTCGTTACATGGA
 TGCACAGTCGCCAGTCCAATGAACATTGAAGTGAGCGAACTGTTTCGCTTC
GGTGGCAGTACTACTCAAAGAATGAGCTGCTGTTAAAAATGCACTCTCGT
TCTCTCAAGTGAGTGGCAGATGAGTGCTCACGCCTTGCACTTCGCTGCCCG
 45 TGTCATGCCCTGCGCCCCAAAATTTGAAAAAAGGGATGAGATTATTGGGC
AATGGACGACGTCGTCGCTCCGGGAGTCAGGACCGGCGGAAAATAAGAG
GCAACACACTCCGCTTCTTA**gctcttc**

[0214] Additional transforming constructs to test the activity of LPAATs from *B. napus*, *T. cacao*, *G. hombroiana* and *G. indica* contained the same selectable marker, restriction sites, promoters and 3' UTR elements as pSZ4198. The coding sequences of *BnLPAT2*(*Bn1.5*), *TcLPAT2*, *GhomLPAT2A*, *GhomLPAT2B*, *GhomLPAT2C*, *GindLPAT2A*, *GindLPAT2B* and *GindLPAT2C* are shown in below. In each case the initiator ATG and terminator TGA are indicated by uppercase italics; the sequence encoding the *LPAT2* homolog is represented by lowercase italics. The *Brassica napus* LPAAT2(BN1.13) sequence is from Genbank accession GU045435. The *Theobroma cacao* LPAAT2 sequence is from the cocoaGenDB database.

10

[0215] SEQ ID NO: 89 Nucleotide sequence of the BnLPAT2(1.5) coding sequence, used in the transforming DNA from pSZ4202

ATGgccatggccgcccgcgcgtgatcgtgccccctgggcaatcctgttcttcaatccggcctgggtggggaacctgctgc
aggccgtgtgctacgtgctgatccgccccctgtccaagaacacctaccgcaagatcaaccgcgtgggtggccgagacc
ctgtggctggagctgggtgtggatcgtggactgggtgggcccggcggtgaagatccagggtgttgcgcgacgacgagaccttc
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gccgcgacaccttccccggccagaaggagcacacaatcggccgccccatcaagtccttggccgtgggtgtcctg
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cgccctgggcccgtgggcacatcaacctgtgcatgcagatcctgatccgctcctcccagtcgcgagcgtccacccccgcc
aagggtggcccccgccaagcccaaggacaagcaccagtcggctcctcctccagaccgaggtggaggagaagca
gaagTGA

30 [0216] SEQ ID NO: 90 Nucleotide sequence of the TcLPAT2 coding
sequence, used in the transforming DNA from pSZ4206

35 *ATGgccatgcgcgcgcgcgcgtgatcgtgccctgggcctgctgttcttcatctccggcctggtggtgaacctgatccaggccct
gtgcttcgtgctgatccgccccctgtccaagaacacctaccgcaagatcaaccgcgtggtggccgagctgctgtggtggagct
gatctggctggtggactggtggggccgcgtgaagatcaagggttcattggaacccgagtccttcaacctgatgggcaaggag
cacgcctggtggtggccaaccaccgctccgacatcgactggctggtgggctggtgctggtggccagcgctccggctgctgggc
tcgcctggcgcgtgatgaagaagtctccaagttctgcccgtgatcgctggtccatgtggttctccgagtacctgttctgga
gcgctcctgggccaaggacgagaacacctgaaggccggcctgcagcgctgaaggactccccgcccttctggtggtgctt
cttcgtggagggcacccgcttaccacaggccaagtctctggcgcgcaggagtagcgcgcctccaggggcctgccatccccgc
aacgtgctgatccccgcaccaaggcttcgtgtccgcctgtccacatgcgctccttcgtgccgccatctacgacatgacct*

ggccatcccaagtctccccctccccaccatgtgcgcctgttcaagggccagccctccgtggtgcacgtgcacatcaagcgct
 gcctgatgaaggagctgcccagaccgacgaggccgtggccagtggtgcaaggacatgttcgtggagaaggacaagctgc
 tggacaagcacatcgccgaggacaccttctccgaccagcccatgcaggacctgggcccctcaagtcctgtggtggtg
 5 gctcctgggctgctgatggcctacggcgccctgaagttcctgcagtgtcctcctgtgtcctcctggaagggcatcgcttc
 ttctggtgggctggccatcgtgaccatcctgatgcacatcctgatcctgttctcccagtcggagcgctccacccccgcaaggt
 ggccccggcaagcccaagaacgacggcgagacctccgaggcccgccgcgacaagcagcagTGA

[0217] SEQ ID NO: 91 Nucleotide sequence of the GhomLPAT2A coding sequence, used in the transforming DNA from pSZ4412.

10 ATGgccatccccgcccatcgtgatcgtgcccggtgggctgctgttcttcatctccggcctgatcgtgaacctgctgcaggccct
 gtgcttcgtgctgatccgccccctgtccaagtcgcctaccgacccatcaaccgccagctggtggagctgctgtggctggagctg
 gtgtgcatcgtggactggtggggcggtgaagatccagctgttcaccgacaaggagacctgaactccatgggcaaggagc
 acgcctggtgatgtgcaaccaccgctccgacatcgactgggtggtgggctggatcctggccagcgctccggtgctgggct
 ccaccgtggcgtgatgaagaagtctccaaggtgctgcccgtgatcggtggtccatgtggttctccgagtacctgttcttggg
 15 gcgcaactgggccaaggacgagtcacccctgaagtccggcctgcagcgctgcgcgacttccccgcccccttctggctggccct
 gttcgtggagggcacccgcttcaccagcccaagctgctggcgcccaggagtacgccgctccaccggcctgcccatcccccg
 caactgctgatccccgcaccaagggcttcgtgctccgctgtccatcacccgctccttcgtgcccgtgatctacgacatcacccg
 tggccatcccaagtctccccagccaccatgctgcgcctgttcaagggccagtcctccgtggtgcacgtgcacctgaagcg
 ccacctgatgaaggacctgcccagtcggacgacgacgtggccagtggtgcccgcgaccagttcgtggtgaaggactccctgc
 20 tggacaagcacatcgccgaggacaccttctccgaccagagctgcaggacatcgccgcccccatcaagtcctggtggtgttc
 acctcctgggtgtgcatcatcaccttcggcgccctgaagttcctgcagtgggtcctcctgtgctactcctggaagggcatcgccat
 ctccgctccggcctggccatcgtgacctgctgatgcacatcctgatccgcttctcccagtcggagcactccacctccgccaaga
 tcgccgaggagaagcacaagaacggcggtgtcccaggagatgggcccgcgagaagcagcacTGA

[0218] SEQ ID NO: 92 Nucleotide sequence of the GhomLPAT2B coding sequence, used in the transforming DNA from pSZ4413.

ATGgagatccccgccgtggccgtgatcgtgcccatcgccatcctgttcttcatctccggcctgatcgtgaacctgatgcaggcca
 tctgcttcttctgatccgccccctgtccaagaacaccaccgcatcgtgaaccgccagctggccgagctgctgtggctggagctg
 atctggatcgtggactggtggggcggtgaagatccagctgttcaccgacaaggagacctgcacctgatgggcaaggagc
 30 acgcctggtgatctgcaaccactcctccgacatcgactgggtggtgggctggctgctgtgcccagcgctccggctgctgggctc
 cgccctggcgtgatgaagtcctcctcaaggtgctgcccgtgatcggtggtccatgtggttctccgagtacctgttcttggagc
 gctcctgggccaaggacgagtcacccctgaagtccggcctgcagcgctgaaggacttccccgcccccttctggctggccctgtt
 cgtggagggcacccgcttcaccagggccaagctgctggcgcccaggagtacgcatgtccgcccgcctgcccgtgccccgca
 acgtgctgatccccgcaccaagggcttcgtgtccgctgttccaacatgcgctccttcgtgcccgccatctacgacgtgacctg
 35 gccatcccaagtctccgtgcagcccaccatgctgcgcctgttcaagggccagtcctccgtggtgcaggtgcacctgaagcgcc
 actccatgaaggacctgcccagtcggaggacgacgtggccagtggtgcccgcgaccgcttcgtggtgaaggactccctgctg
 gacaagcacaaggtggaggacaccttcaccgaccagagctgcaggacctgggcccctcaagtcctggtggtggtga
 cctgctggcctgcatcatcatcttcggcatcctgaagttcctgcagtgggtcctcctgtgtactcctggaagggcatggccatct
 ccgctccggcctggcgtggtgaccttctgatgcagatcctgatccgcttctcccagtcggagcgctccacccccgccaagatc
 40 gccccgccaagcccaacaaggccggcaactcctccgagaccgtgcgcgacaagcaccagTGA

[0219] SEQ ID NO: 93 Nucleotide sequence of the GhomLPAT2C coding sequence, used in the transforming DNA from pSZ4414.

ATGgccatccccgccgcatcatcatcggtgccccctgggcctgatcttcttccacctccggcctgatcatcaacctgatccaggccgt
 gtgtacgtgctgatccgccccctgtccaagtccaccttccgccgcatcaaccgcgagctggccgagctgtgtggctggagctg
 gtgtgggtgggtgactgggtggccggcggtgaagatccagctgttaccgacaaggagaccctgcactccatgggcaaggagc
 acgccctgggtgatctgcaaccaccgctccgacatcgactggctgggtggctggatcctggcccagcgctccggctgcctgggctc
 5 cgccctggccgtgatgaagaagtcctccaaggtgctgccccgtgatcggtgggtccatgtggttctccgagtacttcttctggagc
 gcaactgggcatggacgagtcacccctgaagtcggcctgcagcgctgaaggacttccccagcccttctggctggccctgtt
 cgtggagggcaccgcttaccagcccaagctgctggccgcccaggagtacgcccctccgcccgcctgccatcccccgca
 cgtgtgatcccccgaccaagggcttctgttccgctgaacatcatgcgtccttctgcccgccatctacgacgtgaccgtgg
 ccatcccaagtcctccccagcccaccatgtgctgctgttcaagggccagtcctccgtggtgcacgtgcactgaagcgcca
 10 cctgatggaggacctgcccagaccgacgacgacgtggccagtggtgcccgcgaccgcttctgtggtgaaggactccctgctgg
 acaagtagtggccgaggacaccttctccgaccaggagctgcaggacctggccgccccatcaagtcctggtggtggtgacc
 tctgggtgtgatcatcgcttccgctccctgaagttctgcagtggtcctcctgctgtactcctggaagggcatcgtgatctcc
 gccgctccctggcgtggtgacctgctgatgcagatcctgatccgcttctcccagtcggagcgctccacctccgccaagatcg
 cgccgccaagcgcaagaacgtgggcgagcacTGA

15 **[0220] SEQ ID NO: 94 Nucleotide sequence of the GindPAT2A coding
 sequence, used in the transforming DNA from pSZ4415.**

ATGgccatccccgtggtggtggtgatcggtgccccctgggcctgctgttcttcatctccggcctgatcgtaacctgctcagggccct
 gtgcttctgtgatccgccccctgtccaagtccgctaccgcacccatcaaccgccagctggtggagctgtgtggctggagctg
 gtgtgcatcggtgactgggtggcccgctgaagatccagctgttcatcgacaaggagaccctgaactccatgggcaaggagc
 20 acgccctgggtgatgtgaaccaccgctcctacatcgactggctgggtgggtggatcctggcccagcgctccggctgcctgggctc
 caccgtggccgtgatgaagaagtcctccaaggtgctgccccgtgatcggtgggtccatgtggttctccgagtacctgttctggag
 cgcaactgggccaaggacgagtcacccctgaagtcggcctgcagcgctgcgcgacttccccgccccttctggctggccctgt
 tctgtggagggcaccgcttaccagcccaagctgctggccgcccaggagtacgcccctccaccggcctgccatcccccgca
 acgtgctgatccccgcaccaagggcttctgttccgctgtccatcccgctccttctgtgcccgtgatctacgacatcaccgtg
 25 gccatcccaagtcctcctccagcccaccatgctgaagctgttcaagggccagtcctccgtggtgcacgtgcacctgaagcgcc
 acctgatgaaggacctgcccagtcggacgacgacgtggccagtggtgcccgcgcccagttcgtggtgaaggactccctgctg
 gacaagcacatcgccgaggacaccttctccgaccaggagctgcaggacatcgcccggcccatcaagtcctggtggtgttac
 ctctgggtgtgatcatcaccttggcgccctgaagttctgcagtggtcctcctgctgcactcctggaagggcatcgccatctc
 cgctccggcctggccatcgtgacctgctgatgcacatcctgatccgcttctcccagtcggagcactccacctccgccaagatcg
 30 ccgcccgaagcacaagaacggcgcgctgtcccaggagatgggcccgcgagaagcagcacTGA

**[0221] SEQ ID NO: 95 Nucleotide sequence of the GindPAT2B coding
 sequence, used in the transforming DNA from pSZ4416.**

ATGggcatccccgccgtggccgtgatcggtgccccatcgccatcctgttcttcatctccggcttcatcgtaacctgatgcaggccat
 ctgcttctgtgatccgccccctgtccaagaacacctaccgcacgtgaaccgccagctggccgagttcctgtggctggagctg
 35 atctgggtggtgactgggtggccggcggtgaagatccagctgttaccgacaaggagaccctgcactgatgggcaaggagc
 acgccctgggtgatctgcaaccaccgctccgacatcgactggctgggtgggtggctgctgtgccagcgctccggctgcctgggctc
 cgccctggccgtgatgaagtcctcctccaaggtgctgccccgtgatcggtgggtccatgtggttctccgagtacctgttctggagc
 gctcctgggccaaggacgagtcacccctgaagctggcctgcagcgctgaaggacttccccgccccttctggctggccctgtt
 cgtggagggcaccgcttaccagggccaagctgctggccgcccaggagtacgcatgtccgcccgcctgcccgtgccccgca
 40 acgtgctgatccccgcaccaagggcttctgttccgctgtccaacatgcgctccttctgtgcccgcctactacgacgtgacctg
 gccatcccaagtcctcctgagcccaccatgctggcctgttcaagggccagtcctgctggtgcaggtgcacctgaagcgc
 cacctgatgaaggacctgcccagtcggagggacgacgtggccagtggtgcccgcgagcgcttctgtggtgaaggactccctgct
 ggacaagcacaaggtggaggacaccttctccgaccaggagctgcaggacctgggcccggcccatcaagtcctggtggtggtg
 atctcctgggctgcatcctgatcttctggatcctgaagttctgcagtggtcctcctgctgtactcctggaagggcatcgccatct
 45 ccgctgcgcatggccgtgatcgcttctgatgcagatcctgctgcgttctcccagtcggagcgctccacccccgccaagatc
 gccccgccaagcccaacaacgcccgcaactcctccgagaccgtgctgcgacaagcaccagTGA

[0222] SEQ ID NO: 96 Nucleotide sequence of the GindPAT2C coding sequence, used in the transforming DNA from pSZ4417.

ATGgccatccccgccgcatcatcatcggtccctgggctgatcttcttcacctccggcttcatcatcaacctgatccaggccgt
 gtgtacgtgctgatccgccccctgtccaagtccacctccgccgcatcaaccgccagctggcgagctgtgtgggtggagctg
 5 gtgtgggtgggtgactggtggggccggcgatgaagatccagctgttcaccaacaaggagacctgcactccatcggaaggagc
 acgccttggtgatctgcaaccagcgctccgacatcgactggctgggtgggtggatcctggccagcgctccggctgctgggt
 ccgcccgtggcgtgatgaagaagtctccaaggtgctgccgtgatcggtgggtccatgtggttctccgagtacctgttctgga
 gcgcaactgggcatggacgagtccacctgaagtccggctgcagtggctgaaggactccccagcccttctggtggccct
 gttcgtggagggcacccgcttcacccagcccaagctgctggcgcccaggagtacgccgctccggcggcctgcccacccccg
 10 caactgctgatccccgcaccaagggcttcgtgtccgccgtgaacatcatgcgctccttcgtgcccgcgtgtacgacgtgacc
 gtggcatcccaagctctccccagcccacatgctgcgctgttaaggccagctcctcggtgacgtgcacgtgaagc
 gccacctgatggaggacctgcccagaccgacgacgctggccagtggtgccgcgaccgcttcgtggtgaaggactccct
 gctggacaagcacctggccgaggacaccttctccgaccaggagctgcaggacctggccgccccatcaagtccctggtggtg
 tgacctctgggtgtgcatcatgccttcggcgccctgaagttctgcagtggctcctcctgctgtactctggaagggcatcggt
 15 atctccgcccctcctggccgtggtgacctgctgatgcagatcctgatccgcttctccagtcgagcgctccacctccgcaa
 ggtggtggccgagaagcgcaagaacgtggcgagcacTGA

[0223] Constructs D2971, D2973, D2975, D3219, D3221, D3223, D3225, D3227 and D3229, derived from pSZ4198, pSZ4202, pSZ4206, pSZ4412, pSZ4413, pSZ4414, pSZ4415, pSZ4416 and pSZ4417, respectively, were transformed into the
 20 S6573 parent strain. The fatty acid profiles of primary transformants are shown in Table 10. Also shown are the SOS/SSS ratios determined by LC/MS multiple response measurements. Expression of *LPAT2* genes had no discernable effect on C16:0 or C18:0 accumulation, but C18:2 levels increased by 1-2% compared to the S6573 parent in strains when expressing the D2971, D2973, D2975, D3221, D3223,
 25 and D3227 constructs. Expression of *LPAT2* genes increased C18:2 and also elevated ratios of SOS/SSS, showing reduced accumulation of trisaturated TAGs.

[0224] Table 10. Fatty acid profiles and SOS/SSS ratios of D2971, D2973, D2975, D3219, D3221, D3223, D3225, D3227 and D3229 primary transformants.

Strain	<i>LPAT2</i> gene	SOS/SSS	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 α	C20:0	saturates
S5100			0.7	17.7	4.1	68.5	6.8	0.6	0.4	23.3
S6573.1		15	0.8	6.2	50.7	33.7	5.6	0.7	1.5	59.8
D2971.1	BnLPAT2(1.13)	23	0.8	6.1	51.8	30.5	8.6	0.6	1.4	60.2
D2971.2		16	0.8	6.1	54.3	28.9	7.0	0.6	1.5	63.3
D2971.4		16	0.8	6.4	53.3	29.5	7.3	0.6	1.4	62.6
S6573.2		14	0.8	6.6	52.8	31.7	5.2	0.6	1.5	62.3
D2973.2	BnLPAT2(1.5)	22	0.8	6.2	53.8	28.3	6.4	0.6	1.7	62.7
D2973.38		23	0.9	7.5	51.2	29.1	6.5	0.5	1.4	61.7
D2973.24		24	0.9	6.8	51.7	29.2	6.3	0.5	1.6	61.5
S6573.3		14	0.8	6.6	52.8	31.7	5.2	0.6	1.5	62.3

D2975.33	TcLPAT2	27	0.8	6.6	52.7	29.7	7.1	0.6	1.5	62.3
D2975.13		32	0.8	6.5	52.4	30.2	7.3	0.6	1.4	61.7
D2975.35		27	0.8	6.5	52.8	29.6	7.3	0.6	1.5	62.2
S6573.4		12	0.9	6.4	54.9	28.9	5.7	0.6	1.7	64.5
D3219.19	GhomLPAT2A	12	0.9	7.1	52.4	31.2	4.8	0.5	2.0	63.1
D3219.20		14	0.9	6.6	53.2	30.6	5.5	0.6	1.7	63.0
D3219.32		15	0.8	6.4	53.1	29.8	6.5	0.6	1.5	62.6
S6573.5		12	0.9	6.4	53.7	30.3	5.5	0.6	1.6	63.3
D3220.1	GhomLPAT2B	27	0.9	6.6	52.2	30.0	7.0	0.7	1.4	61.9
D3221.39		20	0.9	6.7	53.9	28.7	6.7	0.6	1.5	63.7
D3221.40		22	0.8	6.5	53.7	29.1	6.8	0.6	1.4	63.2
S6573.6		14	0.8	6.3	54.0	30.2	5.5	0.6	1.6	63.4
D3223.2	GhomLPAT2C	20	0.8	6.5	53.0	29.3	7.3	0.6	1.5	62.4
D3223.6		21	0.8	6.5	53.5	29.3	7.0	0.6	1.4	62.7
D3223.7		21	0.8	6.4	52.5	30.7	6.6	0.5	1.5	61.8
D3225.5	GindLPAT2A	13	0.9	6.6	53.5	30.2	5.6	0.6	1.6	63.2
S6573.7		12	0.9	6.5	53.5	29.9	5.7	0.6	1.8	63.3
D3227.6	GindLPAT2B	23	0.8	6.4	54.1	28.8	6.8	0.6	1.6	63.5
D3227.3		21	0.8	6.5	53.9	29.0	6.7	0.6	1.5	63.4
D3227.17		22	0.8	6.6	53.8	28.8	7.0	0.6	1.4	63.3
S6573.8		11	0.8	6.4	54.3	30.1	5.4	0.6	1.7	63.8
D3229.41	GindLPAT2C	11	0.9	6.6	54.2	29.7	5.6	0.6	1.7	63.9
D3229.27		13	0.8	6.4	54.1	30.0	5.6	0.6	1.7	63.6
D3229.33		12	0.8	6.4	54.0	30.2	5.5	0.6	1.7	63.5

[0225] Table 11 presents the TAG composition of the lipids produced by D2971, D2973, D2975, D3221, D3223, and D3227 primary transformants relative to the S6573 parent. SOS levels in the *LPAT2*-expressing strains were equivalent or slightly higher than in the S6573 controls. Trisaturates declined by up to 53%, and total Sat-
 5 Unsat-Sat levels improved in all of the strains expressing heterologous *LPAT2* genes. Among the *LPAT2* genes, the strains expressing the *T. cacao* *LPAT2* homolog showed the greatest improvements in their TAG profiles).

[0226] Table 11. TAG composition of D2971, D2973, D2975, D3221, D3223, and D3227 primary transformants relative to the S6573 parent.

LPAAT gene		BnLPAT2 (1.13)	BnLPAT2 (1.5)	TcLPAT2		Ghom LPAT2B		Ghom LPAT2C	Gind LPAT2B	
Strain		D2971.1	D2973.38	D2975.33	D2975.13	D3221.39	D3221.40	D3223.6	D3227.3	D3227.6
%S6573 TAG	SOS	100	100	110	104	107	107	108	103	105
	Sat-Sat-Sat	57	63	48	47	74	62	68	62	70
	Sat-U-Sat	109	107	113	110	112	112	109	108	107
	Sat-O-Sat	97	100	105	102	106	105	102	104	104

	Sat-L-Sat	174	147	155	155	139	143	141	130	125
	U-U-U/Sat	85	86	72	83	64	69	78	82	79

[0227] We analyzed the fatty acid profiles, TAG profiles and lipid titers from 50 mL shake flask cultures of stable lines generated from D2975-33. C18:0 and C16:0 levels were comparable between the strains and the S6573 control, and lipid titers ranged from 75-105% of the parent strain titer (Table 12). C18:2 levels increased by more than 2% in the *TcLPAT2*-expressing strains.

[0228] Table 12. Fatty acid profiles of *TcLPAT2*-expressing stable lines made from D2975-33.

Primary		D1940.19	D2975.33				
Strain		S6573	S7813	S7815	S7816	S7817	S7819
Fatty Acid Area %	C12:0	0.2	0.2	0.2	0.2	0.2	0.2
	C14:0	0.9	0.7	0.8	0.8	0.7	0.7
	C16:0	6.5	5.9	6.1	5.9	6.1	6.0
	C16:1 cis-9	0.1	0.1	0.1	0.1	0.1	0.1
	C17:0	0.2	0.2	0.2	0.2	0.2	0.2
	C18:0	56.1	55.6	55.9	56.2	53.9	53.9
	C18:1	28.1	26.8	26.6	26.5	28.8	28.4
	C18:2	5.5	8.1	7.7	7.9	7.7	7.8
	C18:3 α	0.6	0.5	0.6	0.5	0.6	0.7
	C20:0	1.5	1.5	1.4	1.3	1.3	1.5
	C22:0	0.2	0.2	0.1	0.1	0.1	0.2
	C24:0	0.1	0.1	0.1	0.1	0.1	0.1
	saturates	65.7	64.4	65.0	64.9	62.8	62.9

[0229] The TAG profiles of S6573 and S7815 are compared in FIG 1. SOS levels in the *LPAT2*-expressing strains were higher than in the S6573 control. Trisaturates were reduced from 10.2% in S6573 to 5.6% in S7815. Much of the improvement in total sat-unsat-sat levels in S7815 came from a 4% increase in stearate-linoleate-stearate (SLS) and a 1.5% increase in palmitate-linoleate-stearate (PLS), consistent with the enhanced C18:2 content of that strain. These results indicate that the *T. cacao LPAT2* reduces the incorporation of saturated fatty acids at the *sn*-2 position.

[0230] The performance of S7815 versus the S6573 parent strain was compared in high-density fermentations. The fatty acid profile of each strain at the two time points of the fermentations are shown in Table 13. The strains had very similar composition,

with 5.5-5.7% C16:0, 56.4-56.8% C18:0, and 27.2-28.6% C18:1 as the major fatty acids. As was observed in the shake flask assays, (see Table 12), C18:2 levels increased from 5.5% in S6573 to 7.7% in S7815 (Table 13). Normalized lipid titers and yields were comparable between the two strains, indicating that expression of the

5 *TcLPAT2* gene in S7815 did not have deleterious effects on growth or lipid accumulation.

[0231] Table 13. Fatty acid profiles of S7815 versus S6573 fermentations.

Strain		S6573		S7815	
Fermentation		140207F25		140208F26	
Fatty Acid Area %	C12:0	0.19	0.20	0.20	0.21
	C14:0	0.71	0.72	0.66	0.66
	C16:0	5.69	5.73	5.57	5.54
	C16:1 cis-7	0.05	0.05	0.05	0.06
	C16:1 cis-9	0.07	0.06	0.05	0.05
	C17:0	0.11	0.11	0.12	0.11
	C18:0	56.01	56.78	55.50	56.37
	C18:1	29.31	28.58	27.92	27.19
	C18:2	5.56	5.51	7.75	7.70
	C18:3 α	0.34	0.32	0.40	0.37
	C20:0	1.51	1.50	1.35	1.34
	C22:0	0.16	0.16	0.14	0.14
	C24:0	0.10	0.09	0.09	0.08
	sum C18	91.22	91.19	91.57	91.63
	saturates	64.54	65.34	63.69	64.51
	unsaturates	35.46	34.64	36.30	35.49

[0232] Table 13 compares the TAG profiles of the lipids produced during high-density fermentation of S7815 versus S6573. SOS and Sat-Oleate-Sat levels were almost identical between S7815 and the S6573 control. However, Sat-Linoleate-Sat levels increased by more than 7%, and di-unsaturated and tri-unsaturated TAGs (U-U-U/Sat) declined by more than 3% in S7815 compared to S6573. Trisaturates at the end points of the fermentations were reduced from 10.1% in S6573 to 6.1% in S7815. These results indicate that the activity of *T. cocoa* LPAT2 drives the transfer of

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unsaturated fatty acids towards the *sn*-2 position and discriminates against the incorporation of saturated fatty acids at *sn*-2.

EXAMPLE 6: IDENTIFICATION AND EXPRESSION OF NOVEL LPAAT, GPAT, DGAT, LPCAT AND PLA2 WITH SPECIFICITY FOR MID-CHAIN FATTY ACIDS

[0233] In this example, we demonstrate the effect of expression of LPAAT, GPAT, DGAT, LPCAT and PLA2 enzymes involved in triacylglycerol biosynthesis (in previously described *P. moriformis* (UTEX 1435) transgenic strains, S7858 and S8174. S7858 and S8174 were prepared according to co-owned WO2015/051319, herein incorporated by reference. In addition co-owned WO2010/063031 and WO2010/063032 teach the expression *Cuphea hookeriana* *FATB2*. Briefly, strain S7858 is a strain that express sucrose invertase and a *Cuphea. hookeriana* *FATB2*. To make S7858, the construct pSZ4329 (SEQ ID NO: 197) was engineered into S3150, a strain classically mutagenized to increase lipid yield. The plasmid, pSZ4329 is written as THI4a::CrTUB2-ScSUC2-PmPGH:PmAcp-P1p-CpSAD1tp_trimmed_ChFATB2_FLAG-CvNR::THI4a The annotation of the coding portions of pSZ4329 is shown in the Table A below.

[0234] **Table A**

pSZ4329	Identity	Nucleotide Number	Nucleotide Number	Nucleotide Length
THI4a 3' flank	3' flanking sequences of endogenous THI4	5,692	6,394	703
CvNR	3'UTR	5,278	5,679	402
ChFATB2	CDS	4,105	5,271	1,167
CpSAD1tp-trimmed	CDS	3,991	4,104	114
PmAcp-P1 promoter	promoter	3,411	3,981	571
Buffer DNA		3,199	3,404	206
UTR04424=PmPGH UTR	3'UTR	2,749	3,192	444
ScSUC2(o)	CDS	1,144	2,742	1,599
CrTUB2 promoter	promoter	820	1,131	312
THI4a 5' flank	5' flanking sequences of endogenous THI4	27	813	787

[0235] Strain S7858, accumulates C8:0 fatty acids to about 12% and C10:0 fatty acids to about 22-24%. Briefly, strain S8174 is a strain that express sucrose invertase and a *Cuphea. Avigera var. pulcherrima* *FATB2*. To make S8174, the construct pSZ5078 (SEQ ID NO: 198) was engineered into S3150, a strain classically mutagenized to increase lipid yield. pSZ5078 is written as

THI4a5':::CrTUB2_ScSUC2_PmPGH:PmAMT3_CpSAD1tp_trimmed-CaFATB1_Flag_CvNR::THI4a3'. Strain S8174 accumulates C8:0 fatty acids to about 24% and C10:0 fatty acids to about 10%. The annotation of the coding portions of pSZ5078 is shown in the Table B below.

5 **[0236] Table B**

pSZ5078	Identity	Nucleotide Number	Nucleotide Number	Nucleotide Length
THI4a 3' flank	3' flanking sequences of endogenous THI4	6,200	6,902	703
CvNR	3'UTR	5,786	6,187	402
CaFATB1 wild-type	CDS	4,602	5,771	1,170
CpSAD1tp	CDS	4,488	4,601	114
AMT3	promoter eukaryotic	3,411	4,481	1,071
Buffer DNA	misc_feature	3,199	3,404	206
PmPGH	3'UTR	2,749	3,192	444
ScSUC2(o)	CDS	1,144	2,742	1,599
CrTUB2 promoter	promoter	820	1,131	312
THI4a 5' flank	5' flanking sequences of endogenous THI4	27	813	787

[0237] The pool of acyl-CoAs in the ER can be utilized for the synthesis of TAGs as well as phospholipids and long chain fatty acids. The enzymes involved in the synthesis of TAGS and phospholids actively compete against each other for the same substrates. Acyl-CoAs can associate with lysophosphatidate to form phosphatidate which is converted to phosphatidylcholine (PC) and other phospholipid species. PC can be desaturated by FAD2 and FAD3 enzymes to generate polyunsaturated fatty acids, which can be cleaved by phosphotransferases and reenter the acyl-CoA pool. Acyl-CoAs can also be generated from PC directly by acyl-

10 CoA:lysophosphatidylcholine acyltransferase (LPCAT). LPCAT can also catalyze the reverse reaction to consume acyl-CoA. Removal of fatty acids from PC to form acyl-CoAs can also be catalyzed by phospholipase A₂ (PLA₂). TAG formation in the ER from acyl-CoAs requires action of glycerol phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT) and diacyl glycerol acyltransferase

15 (DGAT).

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[0238] The endogenous *P. moriformis* TAG biosynthesis machinery has evolved to function with the longer chain fatty acids that the strain normally makes. We introduced heterologous acyltransferases and phospholipases from species that naturally accumulate high levels of short chain fatty acids into *Prototheca* to increase accumulation of C8:0 fatty acids. We identified the following plant enzymes in NCBI as shown in Table 14 below.

[0239] Table 14. Genes representing target enzymes identified from higher plants that produce high amounts of C8:0 and C10:0. All these genes were synthesized with codon usage optimized for expression in *Prototheca*.

Species	Gene	Enzyme
<i>Cocos nucifera</i>	CnLPAAT1	LPAAT
<i>Cuphea paucipetala</i>	CpauLPAAT1	
<i>Cuphea procumbens</i>	CprocLPAAT1	
<i>Cuphea painteri</i>	CpaiLPAAT1	
<i>Cuphea hookeriana</i>	ChookLPAAT1	
<i>Cuphea ignea</i>	CigneLPAAT1	
<i>Cuphea avigera</i> var. <i>pulcherrima</i>	CavigLPAAT2	
<i>Cuphea avigera</i> var. <i>pulcherrima</i>	CavigLPAAT2	
<i>Cuphea palustris</i>	CpalLPAAT1	
<i>Cuphea koehneana</i>	CkoeLPAAT1	
<i>Cuphea koehneana</i>	CkoeLPAAT2	
<i>Cuphea procumbens</i>	CprocLPAAT2	
<i>Cuphea PSR23</i>	CuPSRLPAAT2	
<i>Cuphea avigera</i> var. <i>pulcherrima</i>	CavigGPAT9	GPAT
<i>Cuphea hookeriana</i>	ChookGPAT9-1	
<i>Cuphea ignea</i>	CignGPAT9-1	
<i>Cuphea ignea</i>	CignGPAT9-2	
<i>Cuphea palustris</i>	CpalGPAT9-1	
<i>Cuphea palustris</i>	CpalGPAT9-2	
<i>Cuphea avigera</i> var. <i>pulcherrima</i>	CavigDGAT1	DGAT
<i>Cuphea hookeriana</i>	ChookDGAT1-1	
<i>Cuphea avigera</i> var. <i>pulcherrima</i>	CavigLPCAT	LPCAT
<i>Cuphea palustris</i>	CpalLPCAT	
<i>Cuphea paucipetala</i>	CpauLPCAT	
<i>Cuphea schumanii</i>	CschuLPCAT1	
<i>Cuphea avigera</i> var. <i>pulcherrima</i>	CavigPLA2-1	PLA2
<i>Cuphea ignea</i>	CignPLA2-1	
<i>Cuphea procumbens</i>	CprocPLA2-2	
<i>Cuphea PSR23</i>	CuPSR23PLA2-2	

[0240] We made a set of constructs expressing heterologous short chain specific acyltransferases and PLA2s as shown in Table 15. The genes were codon optimized to reflect UTEX 1435 codon usage.

[0241] Table 15. List of constructs transformed into S7858 or S8174

D#	Strain	Construct
D4289	S7858	SAD2-1vD::CpaulLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4290	S7858	SAD2-1vD::CpailLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4291	S7858	SAD2-1vD::CignealLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4292	S7858	SAD2-1vD::CprocLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4293	S7858	SAD2-1vD::ChookLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4404	S7858	SAD2-1vD::CnlLPAAT-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4517	S8174	SAD2-1vD::CavigLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4518	S8174	SAD2-1vD::CavigLPAAT2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4519	S8174	SAD2-1vD::CpailLPAAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4690	S8174	SAD2-1vD::CuPSR23 LPAAT2-1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4728	S8174	SAD2-1vD::CkoelLPAAT-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4729	S8174	SAD2-1vD::CkoelLPAAT2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4730	S8174	SAD2-1vD::CprocLPAAT2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4551/D4683	S8174	SAD2-1vD::CavigGPAT9-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4552/ D4684	S8174	SAD2-1vD::ChookGPAT9-1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4553/ D4685	S8174	SAD2-1vD::CignGPAT9-1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4554/ D4686	S8174	SAD2-1vD::CignGPAT9-2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4724	S8174	SAD2-1vD::CpalGPAT9-1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4725	S8174	SAD2-1vD::CpalGPAT9-2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4549	S8174	SAD2-1vD::CavigDGAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4681	S8174	SAD2-1vD::CavigDGAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4555/D4688	S8174	SAD2-1vD::CavigLPCAT-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4726	S8174	SAD2-1vD::CpalLPCAT-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4556/D4689	S8174	SAD2-1vD::CpaulLPCAT-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4727	S8174	SAD2-1vD::CschuLPCAT1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4732	S8174	SAD2-1vD::CavigPLA2-1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4734	S8174	SAD2-1vD::CignPLA2-1-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4735	S8174	SAD2-1vD::CuPSR23PLA2-2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex
D4736	S8174	SAD2-1vD::CprocPLA2-2-PmATP:PmHXT1-ScarMEL-PmPGK::SAD2Bex

[0242] All the constructs shown in Table 15 can be written as SAD2-1vD::gene of interest-PmATP-PmHXT1-ScarMEL-PmPGK::SAD2B, and were made to target the transforming DNA to the SAD2 locus on the genome, thereby disrupting the expression of at least one allele of the endogenous stearoyl ACP desaturase. Sequences of all the transforming DNAs are provided below. The relevant restriction sites in the construct from 5'-3' are- *Pme* I, *BspQ* I, *Kpn* I, *Xho* I, *Avr* II, *Spe* I, *SnaB* I, *EcoR* V, *Sac* I, *BspQ* I, *Pme* I respectively are indicated in lowercase, bold, and underlined. *Pme* I sites delimit the 5' and 3' ends of the transforming DNA. Bold, lowercase sequences at the 5' and 3' end of the construct represent genomic DNA from UTEX 1435 that target integration to the SAD2 locus via homologous recombination, wherein the SAD2 5' flank provides the promoter for the gene of

interest downstream. The primary construct was made with the previously characterized *CnLPAAT* gene as shown below and all other constructs were made by replacing the *CnLPAAT* gene with other genes of interest using the restriction sites, *Kpn* I and *Xho* I that span the gene on either side. Proceeding in the 5' to 3' direction, the first cassette has the codon optimized *Cocos nucifera* *LPAAT* and the *Prototheca moriformis* ATP synthase (PmATP) gene 3' UTR. The initiator ATG and terminator TGA for cDNAs are indicated by uppercase italics, while the coding region is indicated with lowercase italics. The 3' UTR is indicated by lowercase underlined text. The second cassette containing the selection gene melibiose from *Saccharomyces carlsbergensis* (*ScarMEL1*) is driven by the endogenous HXT1 promoter, and has the endogenous phosphoglycerate kinase (PmPGK) gene 3' UTR. In this cassette, the PmHXT1 promoter is indicated by lowercase, boxed text. The initiator ATG and terminator TGA for the *ScarMEL1* gene are indicated in uppercase italics, while the coding region is indicated by lowercase italics. The 3' UTR is indicated by lowercase underlined text. All the final constructs were sequenced to ensure correct reading frames and targeting sequences.

[0243] SEQ ID NO: 97 pSZX61 Sequence of the transforming DNA expressing *CnLPAAT* downstream of the *SAD2* promoter in the cassette followed by the *ScarMEL1* gene for selection downstream of the PmHXT1 promoter in the second cassette.

gtttaaagccggtcaccaccgcatgctcgtactacagcgacgcaccgcttcgtgatccaccgggtgaacgtagtcct
cgacggaaacatctgggtcgggcctcctgcttgactcccgcccatgccgacaacctttctgctgttaccacgaccaca
atgcaacgcgacacgaccgtgtgggactgatcggttcactgcacctgcatgcaattgtcacaagcgcttactccaattgt
attcgtttgttttctgggagcagttgctcgaccgcccgcgtcccgaggcagcgcgatgacgtgtgcgtggcctgggtgtttc
gtcgaaggccagcaaccctaaatcgaggcgatccggagattgggatctgatccgagtttgaccagatccgccccg
atgcggcacgggaactgcatcgactcggcgcggaaccagctttcgtaaatgccagattggtgtccgatacctggattt
gccatcagcgaaacaagacttcagcagcgagcgtatttggcggcgctgctaccagggttgatacattgccatttctg
tctggaccgctttactggcgagagggtgagttgatgggggtggcaggcatcgaaacgcgctgcatgggtgtgcgtgtc
tgtttccggctgcacgaattcaatagtcggatgggacgaggtagaattgggtgtggcgctcgcgtgcatgcctgccccg
tcgggtgtcatgaccgggactggaatccccctcgacacatcttgctaacgctcccgactctccgaccgcgcgagg
atagactcttggttaaccaatcgacaggtaccATGgacgcctccggcgctcctccttctgcgcgccgctgctggagtcct
gcttaaggcctccttcggctacgtaatgtccagcccaaggacgcccggccagccctcccgccgccccgacgacg
acttctgtggacgacgaccgctggatcacctgctcgtggtgcgcacgcgcgctgcttctgtccatgatggtgaccac
atcgtgtggaacatgatcatgctgctgctgctgcccctacgcccgcacccgagggcaacctgtacggccacgtgacc
ggccgcatgctgatgtgattctgggcaaccatcaccatcgagggtccgagttctcaacaccgcgccatctacatctgca
accacgcctccctggtggacatcttctgatcatgtggctgatcccaaggacgctgaccatcgccaagaaggagatcatc
ggtatccctgttcggccagctgtacgtgctggccaaccaccagcgcatcgaccgctccaaccctccgcccacatcgagtccat

caaggagggtggcccgcgccgtggtgaagaagaacctgtccctgatcatcttccccgagggcaccgcctccaagaccggccgc
 tgtgcccttcaagaagggttcatccacatcgccctccagacccgcctgccatcggtcgatggtgtgaccggcaccacact
 ggctggcgcaagaactccctgcgctgcgccccccccatcacgtgaagtacttccccatcaagaccgacgactggga
 ggaggagaagatcaaccactacgtggagatgatccacgcctgtacgtggaccacctgccgagtcacagaagccctggtg
 5 tccaagggccgcgacgcctccggccgctccaactccTGAttaataactcgagatgtggagatgtaggggtgctgactcgtt
gaggtgggtgtttttttatcgagtgcgcgcgcggaacgggtccctttttatcgaggtgttccaacgcgcaccgcc
ctctaaaacaacccccaccaccactgtcgaccttctggttttatccgccacggcgccccggaggggcgctcgtctggc
cgcgcgggcagctgtatcgccgcgctcgtccaatggtgtgtaatcttggaaagataataatcgatggatgaggaggag
agcgtgggagatcagagcaaggaatatacagttggcacgaagcagcagcgtactaagctgtagcgtgttaagaaagaa
 10 aaactcgctgttaggctgtattaatcaaggagcgtatcaataattaccgaccctatacctttatctccaacccaatcgcg
cctagggtgcggtgagaatcgaaaatgcatcgttttaggttcggagacgggtcaattccctgctccggcgaatctgtcggtc
 aagctggccagtggacaatgttctatggcagcccgcgacatgggcctccgacgcggccatcaggagcccaaacag
 cgtgtcagggtatgtgaaactcaagaggtccctgctgggcactccggccccactccggggcgggacgccaggcattcg
 cggtcggtcccgcgcgacgagcgaaatgatgattcggttacgagaccaggacgtcgtcgaggtcgagaggcagcctcg
 15 gacacgtctcgtagggcaacgccccgagtcgcccgagggcggtaaacattgtttctgggtgtcggagtgggcattttg
 ggccgatccaatcgctcatgccgtctcgtctggtcctcacgttcggtacggcctggatcccgaaagggcggtatgc
 acgtggtgttggcccgccattggcgccacgtttcaaagtccccggccagaaatgcacaggaccggcccggtcgcaca
 ggccatgctgaacgccagatttcgacagcaacacatctagaataatcgcaaccatccgcttttgaacgaaacgaaa
 cggcgtgttagcatgtttccgacatcgtgggggcccgaagcatgctccgggggaggaaagcgtggcacagcggtagc
 20 ccattctgtgccacacgccgacgaggaccaatccccggcatcagccttcacgacgggtcgccgcacatataaagccg
 gacgcctaaccggttctgtggtatgactagtATGttcggttctacttctgacggcctgcatctccctgaagggcggtg
 ttcggcgttccccctctacaacggcctgggcctgacgccccagatgggtgggacaactggaacacgttcgcctg
 cgacgtctccgagcagctgctgtggacacggccgaccgcatctccgacctgggctgaaggacatgggtacaag
 tacatcatcttgacgactgctggtcctccggccgcgactccgacgggttctggtcgccgacgagcagaagttcccc
 25 aacggcatgggccacgtcgccgaccactgcacaacaactccttctgttcggcatgtactctccgcgggcgagta
 cacgtgcgccggtacccccggtccctgggcccgcgaggaggaggacgcccagttcttcggaacaacccggtgga
 ctacctgaagtacgacaactgctacaacaagggccagttcggcacgcccagatctctaccaccgctacaaggcc
 atgtccgacgcctgaacaagacgggcccgcctcttctactccctgtgcaactggggccaggacctgaccttctac
 tggggctccggcatcggaactcctggcgcatgtccggcgacgtcacggcgagttcacgcgccccgactcccgtg
 30 cccctgcgacggcgacgagtacgactgcaagtacggcgttccactgtccatcatgaacatcctgaacaaggcc
 gccccatgggcccagaacgcgggctcggcgggtggaacgacctggacaacctggaggtcggcgtcggaacct
 gacggacgacgaggagaaggcgacttctccatgtgggcatggtgaagtccccctgatcatcggcgcgaacgt
 gaacaacctgaaggcctcctactccatctactccaggcgctcgtcatcgccatcaaccaggactccaacggcat
 ccccgccacgcgctgtggcgctactacgtgtccgacacggacgagtacggccagggcgagatccagatgtggtcc
 35 ggccccctggacaacggcgaccaggtcggtggcgctgtgaacggcggtccgtgtcccgccccatgaacacgaccc
 tggaggagatcttctcgactccaacctgggtccaagaagctgacctccacctgggacatctacgacctgtggcg
 aaccgctcgacaactccacggcgtccgccatcctgggcccgaacaagaccgccaccggcatctgtacaacgcca
 ccgagcagtcctacaaggacggcctgtccaagaacgacacccgcctgttcggccagaagatcggtccctgtcccc
 aacgcgatcctgaacacgaccgtccccgcccacggcatcggttctaccgctgcgccccctctccTGAtacaactta
 40 ttacgtattctgaccggcgctgatgtggcgcgacgccgtcgtactctttcagactttactcttgagggaattgaacctttctc
gcttctggcatgtaaacattggcgcaattaattgtgtgatgaagaaagggtggcacaagatggatcgcaatgtacga
gatcgacaacgatggtgattgtatgaggggccaacctgggtcaatcttctgcgatgtccggcgcaatgtgatccagcg
gctgactctcgcaacctggtagtgtgtgcgcacgggtcgctttgattaaaactgatcgattgccatcccgtcaactca
caagcctactctagctccattcgccactcgggcgcccggtcgatcaatgttctgagcggaggggcgaagcgtcaggaa
 45 atcgtctcggcagctggaagcgcatggaatgcggagcggagatcgaatcagatatcAAGCTCCATCgagctccagc
cacggcaacaccgcgccttgcggccgagcagggcgacaagaacctgagcaagatctcggggtgatcgccagcga

cgagggccggcacgagatcgctacacgcgcacgtgtggacgagttcttcgcctcgacccccgagggcgccgtcgccgc
ctacgccaacatgatgcgcaagcagatcaccatgcccgcgcacctcatggacgacatgggccacggcgaggccaacc
cgggccgcaacctcttcgccgacttctccgcggtcgccgagaagatcgacgtctacgacgccgaggactactgccgcat
cctgggagcacctcaacgcgcgctggaaaggtggacgagcgccaggtcagcgggccaggccgccgaggaccaggagtacg
5 tcttggggctgccccagcgcttcgggaaactcgccgagaagaccgcccaagcgcaagcgctcgcgcgaggcccg
tcgcttctcttgatctccgggcgcgagatcatggtctagggagcgacgagtgctgctgcggggctggcgggagtggg
acgcccctctcgctctctctgttctgaacggaacaatcgggcaccgccgctacgcgccacgcatcgagcaacgaaga
aaacccccgatgataggttgcggtggctgcccggatatagatccggccgcacatcaaagggcccttcgccagagaa
gaagctccttccagcagactcctgaagagcgtttaaac.

10 **[0244]** The sequence for all of the other acyltransferase constructs are identical to that of pSZEX61 with the exception of the encoded acyltransferase. The acyltransferase sequence alone is provided below for the remaining acyltransferase constructs.

15 [0245] SEQ ID NO: 98 *CpauLPAAT1*

20
 25
 30

ggtaccATGgccatcccgccgcgcgcgtgatcttctgttcggcctgctgttcttccatccggcctgatcatcaacctg
 tccaggccctgtgtcttctgtctgtggtgtggccctgtccaagaacgccatccgcgcacatcaaccgcgtgttcgccgagct
 gctgctgtccgagctgtgtgtccctgttcgactgggtggggccggcgccaagctgaagctgttaccgaccccgagaccttc
 cgcctgatgggcaaggagcacgcccgtggtgatcatcaaccacatgaccgagctggactggatgctgggctgggtgat
 gggccagcacctgggtgtccctgggtctccatctgtccgtggccaagaagtcaccaagtcttgcctgtgggtgtgg
 tccatgtgggttctcgagtacctgtacatcgagcgctcctggggccaaggaccgcaccacctgaagtccacatcgagc
 gctgaccgactacccctgtcccttctggatgggtgatcttctgtggagggcacccgcttcacccgcaccaagctgtgtgc
 cgcccagcagtagccgcctctcctgggctgtccgtgtccccgcaacgtgtgatccccgcaccaagggtcttctgtgtc
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 gacctggggcaaccgcgtgaccgacgaggtgcacgccccccaccaccatcaaccgcgcctgaagcgcaacgc
 caccggccccgctgacggcgacaccgagggcgcccgctgcgttccgccccctacgtgtccaagggtgccctggc
 acaccggcttccgcgccgtgctgtcccagctgttccccgctacggccactactgcccgcacaaactggctcctccggcaa
 15 gaacggcggtcccccgctgtgggaccagcgccccatcgactggctggactactgctgtactgccacgacatcggtta
 cgacacccacgaccaggccaagctgctggaggcgacctggccttctggagtgcctggagcgccccctctaccccc
 ccaccggcgacgccccacgtggcccatgtacaagaccatgtgcgtgaccggcctgcgcaacgtgctgatccccctac
 cgcacccagctgctgcgcctgaacttccgccagccccgatcgacttggctggctgtccaacgcgcgctggaagggt
 ggtccgcccagaagaccTGActcgag

20 **[0271] SEQ ID NO: 124 CuPSR23PLA2-2**

ggtaccATGgtgcacctgccccacacctgaagctgggcctgggtgacgccatctccatctccggcctgtgcttctct
 ccacccccgcgcgccccgaacgtgggcatccaggcgccggcggtgacctgtccgtgggcaagggtgctcccg
 aagtgcgagtcgacttctgcaagggtgcccccttctgcgtacggcaagtaactgcggcctgatgtactccggctgcc
 cggcgagaagccctgcgacggcctggacgcctgctgcatgaagcacgacgcctgcgtgcaggccaagaacaaga
 25 ctacctgtcccaggagtgtcccagaacctgctgaactgcatggcctcctccgcatgtccggggcgaagcagttcaag
 ggctccacctgccagggtggacgaggtggtggacgtgctgacctggtgatggaggcgccctgctggccggcgcta
 cctgcacaagcccTGActcgag

[0272] SEQ ID NO: 125 CprocPLA2-2

30 ggtaccATGgtgcacctgccccacacctgaagctgggcctgggtgacgccatctccatctccggcctgtgcttctct
 ccacccccgcgcgccccgaacgtgggcatccaggcgccggcggtgacctgtccgtgggcaagggtgctcccg
 aagtgcgagtcgacttctgcaagggtgcccccttctgcgtacggcaagtaactgcggcctgatgtactccggctgcc
 cggcgagaagccctgcgacggcctggacgcctgctgcatgaagcacgacgcctgcgtgcaggccaagaacgacga
 ctacctgtcccaggagtgtcccagaacctgctgaactgcatggcctcctccgcatgtccggggcgaagcagttcaag
 35 ggctccacctgccagggtggacgaggtggtggacgtgctgacctggtgatggaggcgccctgctggccggcgcta
 cctgcacaagcccTGActcgag

[0273] The constructs containing the codon optimized genes described above driven by the UTEX 1453 SAD2 promoter, were transformed into strain S7858 or S8714.

Transformations, cell culture, lipid production and fatty acid analysis were all carried
 40 out as described herein. The transgenic strains were selected for their ability to grow
 on melibiose. Stable transformants were grown under standard lipid production
 conditions at pH5 (for transgenic strains generated in the strain S7858) or at pH7 (for
 the transgenic strains generated in the strain S8174) for fatty acid analysis.

Expression of LPAATs

[0274] In WO2013/158938 we disclosed that *Cocos nucifera* LPAAT enzymes exhibit chain length specificity for the fatty acid acyl-CoA that it attach to the glycerol backbone. We disclosed the impact of expressing *CnLPAAT* in a transgenic strain also expressing a laurate specific thioesterase. In this example we transformed 5 LPAAT enzymes derived from C8-C10 rich *Cuphea species* and the *CnLPAAT* into S7858, and the remaining 8 LPAAT enzymes were transformed into S8174. The resulting fatty acid profiles from a set of representative transgenic lines arising from these transformations are shown in Tables 16 and 17. Expression of these genes as shown in Table 16 resulted in increases in C8:0 and/or-C10:0 fatty acid accumulation.

[0275] **Table 16: Fatty acid profiles of representative transgenic strains of S7858 expressing optimized versions of the *CpauLPAAT1*, *CpalLPAAT1*, *CignLPAAT1*, *CprocLPAAT1*, *ChookLPAAT1* and *CnLPAAT1*.**

Sample ID	C8:0	C10:0	C12:0	C8-C10
S6165	0.00	0.00	0.05	0.00
S7858	11.70	23.36	0.48	35.06

CpauLPAAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S7858; D4289-7	12.69	25.06	0.51	37.75
S7858; D4289-12	11.98	24.54	0.48	36.52
S7858; D4289-2	11.68	24.14	0.49	35.82
S7858; D4289-13	11.53	24.18	0.49	35.71
S7858; D4289-11	11.47	23.85	0.46	35.32

CprocLPAAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S7858; D4292-15	11.86	24.05	0.46	35.91
S7858; D4292-11	11.49	24.01	0.48	35.50
S7858; D4292-22	11.49	23.81	0.47	35.30
S7858; D4292-3	11.46	23.76	0.46	35.22
S7858; D4292-24	11.38	23.64	0.46	35.02

CpalLPAAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S7858; D4290-3	13.43	25.04	0.52	38.47
S7858; D4290-25	12.98	24.75	0.51	37.73
S7858; D4290-5	12.27	25.00	0.52	37.27
S7858; D4290-12	11.98	24.21	0.48	36.19
S7858; D4290-22	11.91	23.86	0.49	35.77

ChookLPAAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S7858; D4293-4	11.09	24.48	0.51	35.57
S7858; D4293-16	12.03	24.24	0.48	36.27
S7858; D4293-6	11.83	23.79	0.48	35.62
S7858; D4293-2	11.81	23.69	0.47	35.50
S7858; D4293-12	11.65	23.11	0.49	34.76

CignLPAAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S7858; D4291-13	12.95	24.78	0.52	37.73
S7858; D4291-20	12.13	24.63	0.49	36.76

CnLPAAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S7858; D4404-11	12.30	24.31	0.47	36.61
S7858; D4404-6	12.03	24.02	0.46	36.05

S7858; D4291-15	12.12	24.35	0.47	36.47	S7858; D4404-13	11.48	23.98	0.46	35.46
S7858; D4291-22	11.94	24.50	0.47	36.44	S7858; D4404-2	11.54	23.71	0.46	35.25
S7858; D4291-7	12.11	23.14	0.50	35.25	S7858; D4404-1	11.76	23.36	0.48	35.12

[0276] Table 17 Fatty acid profiles of representative transgenic strains of S8174 expressing *CavigLPAAT1*, *CavigLPAAT2*, *CpallLPAAT1*, *CuPSR23LPAAT1*, *CkoeLPAAT1*, *CkoeLPAAT2*, *CprocLPAAT1* and *CprocLPAAT2* before lipase treatment.

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Sample ID	C8:0	C10:0	C12:0	C8-C10	
S7485	0.00	0.00	0.07	0.00	
S8174	24.32	9.24	0.37	33.56	
CavigLPAAT1 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4517-23	25.42	9.63	0.39	35.05	
S8174; D4517-9	25.44	9.61	0.39	35.05	
S8174; D4517-8	25.09	9.84	0.39	34.93	
S8174; D4517-18	25.20	9.65	0.39	34.85	
S8174; D4517-2	25.20	9.57	0.37	34.77	
CkoeLPAAT1 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4728-8	25.44	10.31	0.46	35.75	
S8174; D4728-10	24.15	9.51	0.43	33.66	
S8174; D4728-5	23.88	9.56	0.45	33.44	
S8174; D4728-6	23.58	9.28	0.40	32.86	
S8174; D4728-9	23.47	9.25	0.40	32.72	
CavigLPAAT2 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4518-2	24.25	9.97	0.42	34.22	
S8174; D4518-45	24.09	9.65	0.39	33.74	
S8174; D4518-34	23.94	9.71	0.38	33.65	
S8174; D4518-10	24.11	9.50	0.37	33.61	
S8174; D4518-4	23.93	9.59	0.39	33.52	
CkoeLPAAT2-1 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4729-2	25.20	9.81	0.43	35.01	
S8174; D4729-1	23.49	10.60	0.46	34.09	
S8174; D4729-4	22.25	9.45	0.40	31.70	
S8174; D4729-5	18.24	8.22	0.35	26.46	
CpallLPAAT1 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4519-27	25.06	9.75	0.37	34.81	
S8174; D4519-4	23.05	10.74	0.47	33.79	
S8174; D4519-28	24.11	9.54	0.37	33.65	
S8174; D4519-10	23.57	9.51	0.38	33.08	
S8174; D4519-12	23.55	9.49	0.38	33.04	
CprocLPAAT2 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4730-14	24.97	9.92	0.41	34.89	
S8174; D4730-13	23.26	10.72	0.49	33.98	
S8174; D4730-1	23.79	10.15	0.49	33.94	
S8174; D4730-7	23.42	10.13	0.36	33.55	
S8174; D4730-5	23.69	9.49	0.42	33.18	
CuPSR23LPAAT2-1 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4690-2	25.88	10.62	0.43	36.50	
S8174; D4690-1	24.60	9.82	0.44	34.42	
S8174; D4690-3	24.13	9.62	0.47	33.75	
CuPSR23LPAAT4 @ SAD2-1vD locus					
Sample ID	C8:0	C10:0	C12:0	C8-C10	
S8174; D4731-1	25.94	10.87	0.56	36.81	
S8174; D4731-3	22.79	11.52	0.59	34.31	
S8174; D4731-5	22.89	11.22	0.53	34.11	

S8174; D4690-4	23.38	9.97	0.41	33.35	S8174; D4731-2	22.99	11.07	0.45	34.06
					S8174; D4731-4	21.15	9.63	0.43	30.78

[0277] To assess the regiospecific activity of novel LPAAT enzymes, oil extracted from some of these transformants were treated with porcine pancreatic lipase, which selectively hydrolyzes the fatty acids at the sn-1 and sn-3 positions from the glycerol unit of the triacylglycerol, leaving monoacylglycerols (MAGs) with fatty acids located only at the *sn*-2 position. The resulting mixture of monoacylglycerols (2-MAGs), were isolated by solid phase extraction on an amino propyl cartridge followed by transesterification to generate fatty acid methyl esters (FAMES). The fatty acid profiles of these FAMES, which represent the profile of fatty acids at the *sn*-2 position of the various TAGs, were determined by GC-FID. When compared to the fatty acid profiles from transesterification of the oil without lipase treatment, the *sn*-2 fatty acid profiles show that the expressed LPAAT are selective for the *sn*-2 position.

[0278] The *sn*-2 analyses after lipase treatment disclosed in Table 18 show that *CavigLPAAT1*, *CpaiLPAAT* exhibit selectivity for either C8:0 fatty acids and *CpauLPAAT*, *CignLPAAT* are selective for C10:0 fatty acids, demonstrating that the heterologous LPAATs expressed in these transgenic strains have activities that acylate at the *sn*-2 position with preference for C8:0 or C10:0.

[0279] **Table 18 Fatty acid profiles & *sn*-2 analysis of representative transgenic strains of S7858 & S8174 expressing codon optimized versions of the *CnLPAAT1*, *CpauLPAAT1*, *CpaiLPAAT1*, *CignLPAAT1*, *ChookLPAAT1* and *CavigLPAAT1*, *CavigLPAAT2*, *CpalLPAAT1***

Expression of GPATs, DGATs, LPCATs and PLA2s:

[0281] Table 19a. Fatty acid profiles of representative transgenic strains of S8174 expressing GPATs and DGATs

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S8174; D4551-7	24.24	9.04	0.36	33.28	S8174; D4554-3	24.28	8.90	0.42	33.18
S8174; D4551-2	23.93	8.92	0.37	32.85	S8174; D4554-7	23.86	8.96	0.43	32.82
S8174; D4551-6	23.63	8.92	0.41	32.55	S8174; D4554-8	23.99	8.81	0.39	32.80
S8174; D4551-3	23.35	8.90	0.43	32.25	S8174; D4554-4	23.87	8.78	0.4	32.65
ChookGPAT9-1 @ SAD2-1vD locus					CpalGPAT9-1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10	Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4552-6	23.57	9.00	0.36	32.57	S8174; D4724-6	25.61	9.52	0.39	35.13
S8174; D4552-4	23.62	8.87	0.37	32.49	S8174; D4724-7	24.91	9.36	0.41	34.27
S8174; D4552-9	23.39	8.97	0.40	32.36	S8174; D4724-2	24.43	9.46	0.39	33.89
S8174; D4552-8	23.28	8.80	0.40	32.08	S8174; D4724-5	24.01	9.25	0.39	33.26
S8174; D4552-11	23.18	8.80	0.44	31.98	S8174; D4724-4	24.30	8.93	0.39	33.23
CignGPAT9-1 @ SAD2-1vD locus					CpalGPAT9-2 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10	Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4553-12	25.19	9.42	0.40	34.61	S8174; D4725-5	24.24	10.30	0.48	34.54
S8174; D4685-1	24.33	10.24	0.46	34.57	S8174; D4725-6	24.81	9.29	0.41	34.10
S8174; D4553-15	25.11	9.33	0.41	34.44	S8174; D4725-7	24.35	9.51	0.42	33.86
S8174; D4553-1	24.56	9.50	0.44	34.06	S8174; D4725-8	24.37	9.39	0.40	33.76
S8174; D4553-6	24.74	9.16	0.40	33.90	S8174; D4725-9	24.28	9.29	0.41	33.57

[0282] Table 19b. Fatty acid profiles of representative transgenic strains of S8174 expressing DGATs

Sample ID	C8:0	C10:0	C12:0	C8-C10
S7485	0.00	0.00	0.07	0.00
S8174	24.61	9.10	0.42	33.71
Cavig DGAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4549-7	24.89	9.28	0.36	34.17
S8174; D4549-6	24.53	9.04	0.47	33.57
S8174; D4549-4	23.93	8.99	0.41	32.92
S8174; D4549-1	23.93	8.97	0.38	32.90
S8174; D4549-3	23.76	8.9	0.36	32.66
Chook DGAT1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4550-1	24.67	9.12	0.41	33.79
S8174; D4550-3	24.64	9.06	0.42	33.70
S8174; D4682-1	23.72	9.68	0.5	33.40
S8174; D4682-2	23.49	9.66	0.41	33.15

S8174; D4550-2	22.42	8.81	0.41	31.23
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[0283] Table 20: Fatty acid profiles of representative transgenic strains of S8174 expressing LPCATs and PLA2s

Sample ID	C8:0	C10:0	C12:0	C8-C10
S7485	0.00	0.00	0.07	0.00
S8174	24.61	9.10	0.42	33.71

Cavig LPCAT @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4555-1	26.6	9.38	0.47	35.98
S8174; D4555-3	26.4	9.47	0.39	35.87
S8174; D4688-1	25.95	9.67	0.44	35.62
S8174; D4688-3	25.47	9.89	0.44	35.36
S8174; D4555-2	25.52	9.55	0.36	35.07

Cpau LPCAT @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4556-3	25.55	9.21	0.43	34.76
S8174; D4556-4	25.24	9.46	0.41	34.70
S8174; D4689-7	24.63	9.86	0.43	34.49
S8174; D4556-1	25.18	9.13	0.42	34.31
S8174; D4689-6	24.05	9.89	0.48	33.94

Cpal LPCAT @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4726-4	26.34	9.76	0.41	36.10
S8174; D4726-2	25.92	9.9	0.44	35.82
S8174; D4726-3	26.15	9.62	0.41	35.77
S8174; D4726-5	26.09	9.55	0.41	35.64
S8174; D4726-1	25.64	9.57	0.39	35.21

Cschu LPCAT @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4727-1	26.24	9.95	0.45	36.19
S8174; D4727-7	26.26	9.84	0.42	36.10
S8174; D4727-9	26.13	9.87	0.42	36.00
S8174; D4727-11	25.99	9.97	0.44	35.96
S8174; D4727-16	26.28	9.68	0.44	35.96

Cavig PLA2-1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4732-1	26.31	11.24	0.60	37.55
S8174; D4732-2	25.30	11.88	0.50	37.18
S8174; D4732-3	25.29	11.01	0.48	36.30
S8174; D4732-4	25.30	11.00	0.47	36.30
S8174; D4732-5	25.07	11.20	0.44	36.27

CignPLA2-1 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4734-6	26.39	11.34	0.47	37.73
S8174; D4734-1	26.17	10.90	0.46	37.07
S8174; D4734-5	25.58	11.12	0.57	36.70
S8174; D4734-4	25.48	11.17	0.57	36.65
S8174; D4734-2	24.75	11.32	0.46	36.07

CuPSR23PLA2-2 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4735-5	25.81	11.16	0.44	36.97
S8174; D4735-1	25.95	10.92	0.47	36.87
S8174; D4735-8	25.54	10.91	0.42	36.45
S8174; D4735-7	25.45	10.95	0.44	36.40
S8174; D4735-6	25.51	10.88	0.41	36.39

Cproc PLA2-2 @ SAD2-1vD locus				
Sample ID	C8:0	C10:0	C12:0	C8-C10
S8174; D4736-2	25.60	10.87	0.42	36.47
S8174; D4736-4	25.55	10.76	0.40	36.31
S8174; D4736-3	25.40	10.87	0.36	36.27
S8174; D4736-5	25.45	10.46	0.39	35.91
S8174; D4736-1	24.34	11.06	0.48	35.40

EXAMPLE 7: EXPRESSION OF LPAAT AND/OR DGAT IN *PROTOTHECA* TO PRODUCE HIGH SOS AND LOW TRISATURATED TAGS

[0284] In this example we describe genetically engineered *Prototheca moriformis* strains in which we have modified fatty acid and triacylglycerol biosynthesis to maximize the accumulation of Stearoyl-Oleoyl-Stearoyl (SOS) TAGs, and minimize the production of trisaturated TAGs. Tailored oils from these strains resemble plant seed oils known as “structuring fats”, which have high proportions of Saturated-Oleate-Saturated TAGs and low levels of trisaturates. These structuring fats (often called “butters”) are generally solid at room temperature but melt sharply between 35-40°C.

[0285] High-SOS strains were obtained by three successive transformations beginning with strain S5100, a classically improved derivative, of a wild type isolate of *Prototheca moriformis*, S376. Strain S5100 was transformed with plasmid pSZ5654 to generate strain S8754, which produces an oil with increased stearic acid (C18:0) content, lower palmitic acid (C16:0) and reduced linoleic acid (C18:2cisΔ9,12) content relative to S5100. In turn, strain S8754 was transformed with plasmid pSZ5868 to generate strain S8813, which produces oil with higher C18:0, lower C16:0 and improved *sn*-2 selectivity compared to S8754. Finally, strain S8813 was transformed with plasmids pSZ6383 or pSZ6384 to generate strains S9119, S9120 and S9121, producing oils rich in C18:0 with reduced levels of C18:2cisΔ9,12 and improved *sn*-3 selectivity.

[0286] Construct used for *SAD2* knockout in S5100

The first intermediate strains were prepared by transformation of strain S5100 with integrative plasmid pSZ5654 (SAD2-1vD::PmKASII-1tp_PmKASII-1_FLAG-CvNR:CrTUB2-PmFAD2hpA-CvNR:PmHXT1-2v2-ScarMEL1-PmPGK::SAD2-1vE). The construct targeted ablation of allele 1 of the endogenous *stearoyl-ACP desaturase 2* gene (*SAD2*), concomitant with expression of the *PmKASII* gene encoding *P. moriformis* β-keto-acyl-ACP synthase, and a RNAi hairpin sequence to down-regulate *fatty acid desaturase (FAD2)* gene expression. Deletion of one allele of *SAD2* reduced SAD activity, resulting in elevated levels of C18:0. Overexpression of *PmKASII* stimulated elongation of C16:0 to C18:0, further increasing C18:0. FAD2 is responsible for the conversion of C18:1cisΔ9 (oleic) to C18:2cisΔ9,12 (linoleic) fatty

acids, and RNAi of *FAD2* resulted in decreased C18:2. Thus, the first intermediate strains had higher levels of C18:0 and decreased C16:0 and C18:2 fatty acid levels relative to the S5100 parent. The *Saccharomyces carlsbergensis MEL1* gene, encoding a secreted melibiase served as a selectable marker as part of plasmid

5 pSZ5654, enabling the strain to grow on melibiose.

[0287] The sequence of the pSZ5654 transforming DNA is provided below.

Relevant restriction sites in the construct are indicated in lowercase, bold and

underlining and are 5'-3' *PmeI*, *SpeI*, *AscI*, *Clal*, *SacI*, *AvrII*, *EcoRV*, *EcoRI*, *SpeI*,

BsiWI, *XhoI*, *SacI*, *KpnI*, *SnaBI*, *BspQI* and *PmeI*, respectively. *PmeI* sites delimit the

10 5' and 3' ends of the transforming DNA. Bold, lowercase sequences represent *SAD2-1* 5' genomic DNA that permit targeted integration at the *SAD2-1* locus via

homologous recombination. Proceeding in the 5' to 3' direction, bold, lowercase

sequences represent *SAD2-1* 5' genomic DNA sequences that permit targeted

integration at the *FATA-1* locus via homologous recombination. The initiator ATG of

15 the sequence encoding the *P. moriformis* KASII-1 transit peptide (*PmKASII-1tp*) is

indicated by uppercase, bold italics, and the *PmKASII-1tp* sequence located between

the ATG and the *AscI* site is indicated with lowercase, underlined italics. The

PmKASII-1 coding region is indicated by lowercase italics. A sequence encoding a 3X

FLAG tag fused to the C-terminus of *PmKASII-1* is represented by uppercase italics,

20 and the TGA terminator codon is indicated with uppercase, bold italics. The *Chlorella*

vulgaris nitrate reductase (NR) gene 3' UTR is indicated by lowercase underlined

text. A spacer sequence is represented by lowercase text. The *C. reinhardtii* TUB2

promoter, driving expression of the *PmFAD2hpA* sequence is indicated by boxed text.

Bold italics denote the *PmFAD2hpA* sequence followed by lowercase underlined text

25 representing *C. vulgaris* nitrate reductase 3' UTR. A second spacer sequence is

represented by lowercase text. The *P. moriformis* *HXT1* promoter driving the

expression of the *S. carlsbergensis* *MEL1* gene is indicated by boxed text. The initiator

ATG and terminator TGA for *MEL1* gene are indicated by uppercase, bold italics

while the coding region is indicated in lowercase italics. The *P. moriformis* *PGK* 3'

30 UTR is indicated by lowercase underlined text. The *SAD2-1* 3' genomic region

indicated by bold, lowercase text.

atcgacctgcaccgggtgcctacgtgggtcaagtaaggcatcatgtggcgctctactggttctccaggtgtgtt
 gagggttttggtgcccgtattgaggtcctggtggcgcatggaggagaaggcgctgtcccgtgaccccc
 ggctacctcccggcaccttccaggggcggtacgggaagaaccagtagagggccacatgatgccgtacttgacc
 cacgtaggcaccgggtgcaggggtcgtatgtacgtcgacgcgacgtagagcaggacatgaccgcgatgtcaaag
 5 ggcaggtacatgctgctacgaagcgccgagcgctcgaacagtgcgcggggatggccttgcgacgcgtcccgat
cgtgaacggagggttctccacagggtgcctgttcgtcttgatagccatctcagggcagcagcagctcgatagtatcg
acacactctggacgtggtcgtgtgatggactgttgccgccacacttgctgccttgacctgtgaatatccctgccgtttat
caaacagcctcagtggtttgatcttgtgtgtacgcgcttttgcgagttgctagctgcttgtgctatttgcgaataccacccc
cagcatccccttccctgttcatatcgcttgcatcccaaccgcaacttatctacgtgtcctgctatccctcagcgctgtc
 10 ctgctcctgctcactgcccctgcacagccttggtttgggtccgctgtattctcctggtactgcaacctgtaaacgagca
ctgcaatgctgatgcacgggaagtagtgggtgggaacacaaatggaaagctgtagagctcgatctaagtaagattcg
aagcgctcgaccgtgccggacggactgcagccccatgtcgtagtaccgccaatgtaagtgggtggtgttccctgtac
gtgagtcaacgtcactgcacgcgaccacacctcgcaccggcaggaccaggcatcgcgagatacagcgcgagccagac
acggagtccgagctatgcgcacgctccaactaggtaccgctcccgtctggtcctcaggttcgtgtacggcctggatcc
 15 cggaaaggcggtatgcacgtggtgttccccgccattggcgcccacgtttcaaagtccccggccagaaatgcacaggac
cggccccggctcgcacaggccatgacgaatgccagatttcgacagcaaaacaatctggaataatcgcaaccattcgct
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gtggcccaaggtagccattctgtgccacacgccgacgaggaccaatccccggcatcagccttcacgcggtgcgc
cgcacatataaagccggacgccttccgacacgttcaacagttttatttctccacttctgaatcaacaaatcttcaa
 20 ggaagatctgctcttgagcaactcgt**ATG**ttcgcgttctacttctgacggcctgcctcctgaagggcggttgcg
gctctccccctctacaacggcctgggctgacgcccagatgggctgggacaactggaacacgttcgctgcgac
gtctccgagcagctgctgctggacacggccgaccgcatctccgacctgggctgaaggacatgggctacaagtaca
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ggcatgggccaacgtcgcgaccacctgcacaacaactccttctggttcggcatgtactcctccgggcgagtacac
 25 gtgcgccggtacccccggtcctgggcccgcgaggaggagacgcccagttcttcggaacaaccgctggactac
ctgaagtacgacaactgctacaacaagggccagttcggcacgcccagatctcctaccaccgctacaaggccatgt
ccgacgcccgaacaagacgggcccgccttctactcctgtgcaactggggccaggacctgaccttctactgg
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ctgcgacggcgacgagtacgactgcaagtacgcccgttccactgctccatcatgaacatcctgaacaaggccgcc
 30 cccatgggccaagaacgcgggcgctggcggtggaacgacctggacaacctggagggtcggcgctggcaacctgac
ggacgacgaggagaaggcgcaacttctcatgtgggcatggtgaagtccccctgatcatcggcgcgaacgtgaa
caacctgaaggcctcctactccatctactcccaggcgctccgtcatcgccatcaaccaggactccaacggcatcccc
gccacgcgcgtctggcgctactacgtgtccgacacggacgagtacggccaggggcgagatccagatgtggtccggc
cccctggacaacggcgaccaggtcgtggcgctgctgaacggcggtccgtgtcccgcccatgaacacgacctgg
 35 aggagatcttctcgactccaacctgggctccaagaagctgacctccacctgggacatctacgacctgtgggcaac
cgcgtcgacaactccacggcgctccgccatcctgggcccgaacaagaccgccaccggcatcctgtacaacgccaccg
agcagtcctacaaggacggcctgtccaagaacgacacccgcctgttcggccagaagatcggtcctctgtcccca
cgcgatcctgaacacgaccgtccccgccacggcatcggttctaccgctgcgcccctctcc**TGA**tacaacttata
cgtattctgaccggcgctgatgtggcgcgacgccgtcgtactcttccagactttactcttgaggaaattgaaccttctcgt
 40 tgctggcatgtaaacattggcgcaattaattgtgtgatgaagaaagggtggcacaagatggatcgcgaaatgtacgagat
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tgactctcgcaacctggtagtgtgtgcgcaccgggtcgctttgattaaaactgatcgcatcgccatcccgtcaactcaaa
gcctactctagctccattgcgcactcggcgcccggctcgatcaatgttctgagcggaggggcgaagcgtcaggaaatcg
tctcggcagctggaagcgcatggaatgcggagcggagatcgaatcaggatccttagggagcgcagagtgtgcgtgcgg
 45 ggctggcgggagtgaggacgccctcctcgtctctgttctgaacggaacaatcggccaccccgctacgcgcacg

catcgagcaacgaagaaaacccccgatgataggttgcggtggctgccgggatagatccggccgcacatcaaagg
 gccctccgccagagaagaagctcctttccagcagactccttctgctgcaaaacacttctgtccacagcaacacca
 aaggatgaacagatcaacttgcgtctccgctagcttctcggctagcgtgcttgcaacaggtccctgcactattatcttc
 ctgctttctctgaattatgcggcaggcgagcgtcgctctggcgagcgtccttcgcgccccctcgtgatcgagtgtg
 5 cagtcaatgaatggctcctggggaagaacgaggggaatttgggtaaaacaagcatcgtctctcaggccccggcgag
 tggccgttaaagtccaagaccgtgaccaggcagcgagcgcgctccgtgtgctgggcccctgctggggctggcggtgcc
 aggtcgagagcagctccctcaggtcgcttggacggcctctcgaggccggtgagggcctgcaggagcgctcgagc
 gtggcagtggcggtcgatccgggtcgccggtcaccgctcgactcgccatccgaagagcgtttaaac

- 10 [0289] Construct pSZ5654 was transformed into S5100. Primary transformants were clonally purified and screened under standard lipid production conditions at pH 5. Integration of pSZ5654 at the *SAD2-1* locus was verified by DNA blot analysis. The fatty acid profiles and lipid titers of lead strains were assayed in 50-mL shake flasks (Table 21). S8754 was selected as the lead strain for additional rounds of
- 15 genetic engineering. As shown in Table 21, C16:0 decreased from 17.6% to less than 6%, C18:0 increased from 4.3% to about 28%, C18:2 decreased from 5.8% to 1.3%.

[0290] Table 21. Fatty acid profiles of *SAD2-1* ablation strains.

Sample ID	S5100	S8741	S8742	S8743	S8744	S8745	S8746	S8752	S8753	S8754
C14:0	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
C16:0	17.6	5.9	5.9	5.8	5.9	5.9	5.9	5.9	5.8	5.9
C16:1 cis-9	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
C18:0	4.3	28.2	28.1	27.7	27.8	27.4	28.2	28.3	28.3	28.1
C18:1	69.8	60.1	60.2	60.6	60.5	60.9	60.0	60.0	60.0	60.0
C18:2	5.8	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.2	1.3
C18:3 α	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C20:0	0.3	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
saturates	23.2	37.5	37.5	37.1	37.2	36.8	37.7	37.7	37.7	37.6
lipid (g/L)	13.5	12.8	12.5	12.5	12.5	12.3	12.3	12.3	12.4	12.3

20 **Construct used for FATA-1 knockout in S8754**

- [0291] The second intermediate strains were prepared by transformation of strain S8754 with integrative plasmid pSZ5868 (FATA-1vB::CpSAD1tp_*GarmFATA1*(G108A)_FLAG-PmSAD2-1:PmG3PDH-1-TcLPAT2-PmATP:CrTUB2-ScSUC2-PmPGH::FATA-1vC). This construct targeted
- 25 ablation of allele 1 of the endogenous *fatty acyl-ACP thioesterase* gene (*FATA-1*), and contained expression modules for *GarmFATA1*(G108A), encoding a variant of the *Garcinia mangostana* FATA1 thioesterase with improved activity, and *TcLPAT2*

- encoding the *Theobroma cacao* lysophosphatidic acid acyltransferase (LPAAT). Deletion of one copy of *FATA-1* reduced endogenous thioesterase activity, further reducing C16:0 accumulation. Expression of GarmFATA1(G108A) stimulated C18:0-ACP hydrolysis, further increasing C18:0. *TcLPAT2* had superior specificity for
- 5 transfer of C18:1 to the *sn*-2 position of triacylglycerides than the endogenous LPAAT, leading to reduced accumulation of trisaturates. The second intermediate strains had increased C18:0 and lower C16:0 compared their parent, S8754. The *S. cerevisiae* *SUC2* gene encoding a secreted sucrose invertase, served as a selectable marker as part of plasmid pSZ5868 and enabled the strain to grow on sucrose.
- 10 **[0292]** The sequence of the pSZ5868 transforming DNA is provided below. Relevant restriction sites in the construct are indicated in lowercase, bold and underlining and are 5'-3' *BspQI*, *PmeI*, *SpeI*, *AscI*, *ClaI*, *SacI*, *AvrII*, *NdeI*, *NsiI*, *AflIII*, *KpnI*, *XbaI*, *MfeI*, *BamHI*, *BspQI* and *PmeI*, respectively. *BspQI* and *PmeI* sites delimit the 5' and 3' ends of the transforming DNA. Proceeding in the 5' to 3'
- 15 direction, bold, lowercase sequences represent *FATA-1* 5' genomic DNA that permit targeted integration at the *FATA-1* locus via homologous recombination. The initiator ATG of the sequence encoding the *C. protothecoides* SAD1 transit peptide (CpSAD1tp) is indicated by uppercase, bold italics, and the remainder of the *CpSAD1tp* sequence located between the ATG and the *AscI* site is indicated with
- 20 lowercase, underlined italics. The *GarmFATA1(G108A)* coding region is indicated by lowercase italics. A sequence encoding a 3X FLAG tag fused to the C-terminus of *GarmFATA1(G108A)* is represented by uppercase italics, and the TGA terminator codon is indicated with uppercase, bold italics. The *P. moriformis* *SAD2-1* 3' UTR is indicated by lowercase underlined text. A spacer sequence is represented by
- 25 lowercase text. The *P. moriformis* *G3PDH-1* promoter, driving expression of the *TcLPAT2* sequence is indicated by boxed text. The initiator ATG and terminator TGA codons of the *TcLPAT2* gene are indicated by uppercase, bold italics, while the remainder of the coding region is represented with italics. Lowercase underlined text represents the *P. moriformis* *ATP* 3' UTR. A second spacer sequence is represented
- 30 by lowercase text. The *C. reinhardtii* *TUB2* promoter driving the expression of the *S. cerevisiae* *SUC2* gene is indicated by boxed text. The initiator ATG and terminator TGA for *SUC2* are indicated by uppercase, bold italics while the coding region is

indicated in lowercase italics. The *P. moriformis* PGH 3' UTR is indicated by lowercase underlined text. The *FATA-1* 3' genomic region indicated by bold, lowercase text.

[0293] SEQ ID NO: 127 Nucleotide sequence of transforming DNA contained in pSZ5868

5 pSZ5862
gaagagcgcccaatgtttaaacctcttttctgctgctcctcaggcttgggggcctccttgggcttgggtgccgcatgat
ctgcgcgcatcagagaaacgttctggttaaaaaggagcgcccggtgcgcaatatatataggcacgcaacacag
cccaacctcactcgggagcccgtcccaccaccccaagtcgctgccttgacggcatactgctgcagaagcttcatgag
aatgatgccgaacaagagggggcacgaggaccaatcccggacatccttgtcgataatgatctctgtagtccccatcgt
10 ccgcccagcgtccggggagcccgcgatgctcaagacgagagggccctcgaccaggaggggctggccggggcgggc
actggcgtcgaaggtgcgcccgtcgttcgctgcagtcctatgccacaaaacaagtcttctgacgggggtgcgttctcc
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gccatatatttctcacctagcgcgcaaaacctaacattggggagtactgtgccactgagttcgactggtagctgaat
15 ggagtcgctgctccactaaacgaattgtcagcaccgccagccggcgaggaccggagtcatagcgagggtagtagcgc
gccactagtATGgccaccacatccactttctcggcgttcaatgcccgctgcggcgacctgcctcgcgcgggctcc
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20 gtggagaccatcgccaacctgctgcaggaggtgggctgcaaccacgcccagtcctggtgggctactccaccgcccgt
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cccgcctggtccgacgtggtggagatcgagtcctggggccagggcgagggcaagatcggcaccgcgcgactgg
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ggcgctgcagaaggtggacgtggacgtgcgcgacgagtacctggtgactgccccgcgagctgcgctggcctt
25 cccgaggagaacaactcctcctgaagaagatctccaagctggaggaccctcccagtactccaagctgggctg
gtgccccgcgcgacctggacatgaaccagcacgtgaacaacgtgacctacatcggctgggtgctggagtcca
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ctccgccaacgtgtccgccaacgaccacggctgccgcaactcctgcacctgctgcgctgtccggcaacggcctgg
30 agatcaaccgcggccgacccgagtggtgcgaagaagccccccgcATGGACTACAAGGACCACGACGGC
GACTACAAGGACCACGACATCGACTACAAGGACGACGACGACAAGTGAatcgatggagcgacga
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35 agcaacaccaaaggatgaacagatcaacttgcgttccgctagcttctcggctagcgtgcttgcaacaggtccctgca
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 gtggccgagctgctgtggctggagctgatctggctggtggactggtggccggcgtgaagatcaagggtgttcattg
 5 accccgagtccttaacctgatgggcaaggagcacgcctggtggtggccaaccaccgctccgacatcgactggct
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 10 gcaccaagggttcgtgtccgctgtccacatgcgctccttcgtgcccgcctctacgacatgacctggccatccc
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 ttgtcgaccttctcgtttgttatccgccacggcgccccggaggggcgctcgtctggccgcgccggcagctgtatcgccgcg
 20 tgcctccaatggtgtgtaatcttggaaagataataatcgatggatgaggaggagagcgtgggagatcagagcaaggaa
 tatacagttggcacgaagcagcagcgtactaagctgtagcgtgttaagaaagaaaaactcgctgttaggctgtattaatc
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 gcgctcgacctgcccggacggactgcagccccatgtcgtagtaccgccaatgtaagtgggctggcgtttcctgtacgt
 gagtcaacgtcactgcacgcgacaccctctcgaccggcaggaccaggcatcgcgagatacagcgcgagccagacac
 25 ggagtccgagctatgcgcacgtccaact**aggtacc**ctttctgctatgacacttccagcaaaaggtaggcgccgct
 gcgagacggcttccggcgctgcatgcaacaccgatgatgcttcgacccccgaagctccttcggggctgcatggcgct
 ccgatgccgctccaggcgagcgctgtttaaatagccaggcccccgattgcaaagacattatagcgagctaccaaagcc
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 cctcttctgttcagtcacaaccgcaaac**tctaga**atatca**ATG**ctgctgcaggccttctgttctgctggtggcgcttc
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 ggatgaacgacccccacggcctgtggtacgacgagaaggacgccaagtggcacctgtacttcagttacaacccga
 acgacaccgctggtgggacgcccctgttctggggccacgccagctccgacgacctgaccaactgggaggaccagcc
 catcgccatcgccccgaagcgcaacgactccggcgcttctccggtccatggtggtggactacaacaacacctccg
 gcttcttaacgacaccatcgacccgcccagcgctgctggtggccatctggacctacaacacccccggagtcaggga
 35 gcagtacatctctacagcctggacggcggtacaccttaccgagtaccagaagaacccccgtgctggccgccaact
 ccaccagttccgagacccgaaggcttctgtgtacgagccctcccagaagtggatcatgaccgcccgaagtcccag
 gactacaagatcgagatctactcctccgacgacctgaagtctggaagtggagtccgcgttcgccaacgagggct
 tctcgggtaccagtacgagtccccggcctgatcgagggtccccaccgagcaggacccagcaagtctactgggtg
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 20 cccgttctctcctattcttgatccacatgatgaccctggcactatttcaagggtggagaagagcggttaaac

[0294] Construct pSZ5868 was transformed into S8754. Primary transformants
 were clonally purified and screened under standard lipid production conditions at pH
 5. Integration of pSZ5868 at the FATA-1 locus was verified by DNA blot analysis.
 The fatty acid profiles and lipid titers of lead strains were assayed in 50-mL shake
 25 flasks (Table 22). S8813 was selected as the lead strain for the final round of genetic
 engineering. As shown in Table 22 as compared to strain S8754, C16:0 decreased
 from 5.9% to 3.4%, and C18:0 increased from 27.3% to about 45%. C18:2 increased
 slightly from 1.3% to about 1.6% due to the activity of the *T. cacao* LPAAT.

[0295] Table 22. Fatty acid profiles of *FATA-1* ablation strains.

Strain	S5100	S8754	S8813	S8814
C14:0	0.7	0.6	0.5	0.5
C16:0	18.8	5.9	3.4	3.4
C16:1 cis-9	0.5	0.0	0.0	0.0
C18:0	4.0	27.3	45.3	44.8
C18:1	68.3	60.9	45.9	46.3
C18:2	6.3	1.3	1.5	1.6
C18:3 α	0.6	0.3	0.3	0.3
C20:0	0.3	2.4	2.0	2.1
saturates	24.2	37.0	52.0	51.5
lipid (g/L)	12.7	11.9	11.9	11.9

Constructs used for FAD2 knockout in S8813

[0296] The high-SOS strains were generated by transformation of strain S8813 with integrative plasmid pSZ6383 (FAD2-1vA::PmLDH1-AtTHIC-PmHSP90:PmSAD2-2v2-TcDGAT1-CvNR:PmSAD2-1v3-CpSAD1tp_ *GarmFATA1*(G108A)_FLAG-PmSAD2-1::FAD2-1vB), plasmid pSZ6384 (FAD2-1vA::PmLDH1-AtTHIC-PmHSP90:PmSAD2-2v2-TcDGAT2-CvNR:PmSAD2-1v3-CpSAD1tp_ *GarmFATA1*(G108A)_FLAG-PmSAD2-1::FAD2-1vB), or plasmid pSZ6377 (FAD2-1vA::PmLDH1-AtTHIC-PmHSP90: PmSAD2-1v3-CpSAD1tp_ *GarmFATA1*(G108A)_FLAG-PmSAD2-1::FAD2-1vB). These constructs targeted ablation of allele 1 of the endogenous *fatty acid desaturase 2* gene (*FAD2-I*), and contained expression modules for a second copy of *GarmFATA1*(G108A), and either *TcDGAT1* encoding the *Theobroma cacao* diacylglycerol O-acyltransferase 1 (pSZ6383) or *TcDGAT2* encoding the *Theobroma cacao* diacylglycerol O-acyltransferase 2 (pSZ6384). Deletion of one allele of *FAD2* further reduced C18:2 accumulation. Expression of *GarmFATA1*(G108A) stimulated C18:0-ACP hydrolysis, further increasing C18:0. *TcDGAT1* and *TcDGAT2* had superior specificity for transfer of C18:0 to the *sn*-3 position of triacylglycerides than the endogeneous DGAT, leading to an increase in C18:0 and lipid titer, and a reduction in trisaturated TAGs. The final strains had higher C18:0, lower C16:0 and lower C18:2 than their parent, S8813. The *Arabidopsis thaliana* *THIC* gene (*AtTHIC*) catalyzes the conversion of 5-aminoimidazole ribotide (AIR) to 4-amino-5-hydroxymethylpyrimidine (HMP), providing the pyrimidine ring structure for the biosynthesis of thiamine. *AtTHIC* served as a selectable marker as part of plasmids pSZ6383 and pSZ6384, allowing the strains to grow in the absence of exogenous thiamine.

[0297] The sequence of the pSZ6383 transforming DNA is provided below. Relevant restriction sites in the construct are indicated in lowercase, bold and underlined text, and are 5'-3' *BspQI*, *KpnI*, *XbaI*, *SnaBI*, *BamHI*, *AvrII*, *SpeI*, *ClaI*, *AflII*, *EcoRI*, *SpeI*, *AscI*, *ClaI*, *SacI* and *BspQ I*, respectively. *BspQI* sites delimit the 5' and 3' ends of the transforming DNA. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent *FAD2-I* 5' genomic DNA that permits targeted integration at the *FAD2-I* locus via homologous recombination. The *P. moriformis*

LDH1 promoter driving the expression of the *Arabidopsis thaliana THIC* gene is indicated by boxed text. The initiator ATG and terminator TGA for *AtTHIC* are indicated by uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis HSP90* 3' UTR is indicated by lowercase underlined text. A spacer sequence is represented by lowercase text. The *P. moriformis SAD2-2* promoter, driving expression of the *TcDGAT1* sequence is indicated by boxed text. The initiator ATG and terminator TGA codons of the *TcDGAT1* gene are indicated by uppercase, bold italics, while the remainder of the coding region is represented with italics. Lowercase underlined text represents the *C. vulgaris NR* 3' UTR. A second spacer sequence is represented by lowercase text. The *P. moriformis SAD2-1* promoter, indicated by boxed italicized text, is utilized to drive the expression of the *G. mangostana FATA1* gene. The initiator ATG of the sequence encoding the *C. protothecoides* SAD1 transit peptide (*CpSAD1tp*) is indicated by uppercase, bold italics, and the remainder of the *CpSAD1tp* sequence located between the ATG and the *AscI* site is indicated with lowercase, underlined italics. The *GarmFATA1(G108A)* coding region is indicated by lowercase italics. A sequence encoding a 3X FLAG tag fused to the C-terminus of *GarmFATA1(G108A)* is represented by uppercase italics, and the TGA terminator codon is indicated with uppercase, bold italics. The *P. moriformis SAD2-1* 3' UTR is indicated by lowercase underlined text. The *FAD2-1* 3' genomic region is indicated by bold, lowercase text.

[0298] SEQ ID NO: 128 Nucleotide sequence of transforming DNA contained in pSZ6383

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gcggcgcatgacgacctaccacatgcgaagagc

25 **[0299]** The sequence of the pSZ6384 transforming DNA is provided below. Relevant restriction sites in the construct are indicated in lowercase, bold and underlined text, and are 5'-3' *BspQI*, *KpnI*, *XbaI*, *SnaBI*, *BamHI*, *AvrII*, *SpeI*, *ClaI*, *AflIII*, *EcoRI*, *SpeI*, *AscI*, *ClaI*, *SacI* and *BspQ I*, respectively. *BspQI* sites delimit the 5' and 3' ends of the transforming DNA. Proceeding in the 5' to 3' direction, bold, 30 lowercase sequences represent *FAD2-1* 5' genomic DNA that permits targeted integration at the *FAD2-1* locus via homologous recombination. The *P. moriformis* *LDH1* promoter driving the expression of the *Arabidopsis thaliana* *THIC* gene is indicated by boxed text. The initiator ATG and terminator TGA for *AtTHIC* are indicated by uppercase, bold italics while the coding region is indicated in lowercase 35 italics. The *P. moriformis* *HSP90* 3' UTR is indicated by lowercase underlined text. A spacer sequence is represented by lowercase text. The *P. moriformis* *SAD2-2* promoter, driving expression of the *TcDGAT2* sequence is indicated by boxed text. The initiator ATG and terminator TGA codons of the *TcDGAT2* gene are indicated by uppercase, bold italics, while the remainder of the coding region is represented with

italics. Lowercase underlined text represents the *C. vulgaris* NR 3' UTR. A second spacer sequence is represented by lowercase text. The *P. moriformis* SAD2-1 promoter, indicated by boxed italicized text, is utilized to drive the expression of the *G. mangostana* FATA1 gene. The initiator ATG of the sequence encoding the *C. protothecoides* SAD1 transit peptide (CpSAD1tp) is indicated by uppercase, bold italics, and the remainder of the *CpSAD1tp* sequence located between the ATG and the AscI site is indicated with lowercase, underlined italics. The *GarmFATA1(G108A)* coding region is indicated by lowercase italics. A sequence encoding a 3X FLAG tag fused to the C-terminus of *GarmFATA1(G108A)* is represented by uppercase italics, and the TGA terminator codon is indicated with uppercase, bold italics. The *P. moriformis* SAD2-1 3' UTR is indicated by lowercase underlined text. The *FAD2-1* 3' genomic region is indicated by bold, lowercase text.

[0300] SEQ ID NO: 129 Nucleotide sequence of transforming DNA contained in pSZ6384

15 **gctcttcgcaaggtcattttccagaacaacgacatggcttgccttagcgatcgctcgaatgactgctagtgcgtacgctcgacccagtcgctcgcaggagaacgcggcaactgccgagcttcggcttgccagtcgtgactcgtatgtgatcagg**
aatcattggcattggtagcattataattcggcttcgcgctgtttatgggcatggcaatgtctcatgcagtcgaccttagt
caaccaattctgggtggccagctccggcgacccggctccgtgtcgcgggcaccacctcctgcatgagtaacagggc
cgccctctctccgacgttggccaactgaataccgtgtcttggggccctacatgatgggtgccttagtcgggccccgacg
20 **cgcaactgcccgcgcaatctgggacgtggtctgaatctccaggcggtttccccgagaaagaaaggggtgccgatttca**
aagcagagccatgtgccgggccctgtggcctgtgttggcgccatgtagtcacccccctcaccaattgtcgcagttt
gcgcaatccataaactcaaaactgcagcttctgagctgcgctgttcaagaacacctctgggggttgcacccgcgagg
tcgacgggtacc
25 **tcctcgtctctgcactctggcgccctcctcgtctcgtggactgacggacgagagtctgggccccgc**
tttttatccacaccgccctttccgcatcgaagacaccacccatcgctgccgcaggtcttcccaatcacccgccctgtggt
cctctctccagccgtgttggctgctgcgtccacattttccattcgtgccccacgatcctcgccatcttggcgcttggat
aggcaccctttttcagcacgccctggtgtgtagcacaacctgacctctctaccgcatcgctccctccacacctcagt
tgactccctcgtgcacgttgacccgcaagctccccatttcatcctattgacaatcgacactgtacatgtatgtcatta
ttttgcaaaaaaacaggggtcggttactcctggcagacgacgcggtgctgcccgcgcccgtgaggcgcgctgcga
cggcaacacccatcgacccgcacgtcgacgagtaacccacccctgctcaacgggtgatctcccatcgcgacacccccg
30 **tgaccgtactatgtcgtccatacgaacatgaaaaggaccttgggtccccggaggcgagctcgtaatcccagaggtt**
ggccccgcttcgctggacacccatcgcatcttccggctcgccgctgtcgagcaagcgccctcgtgcgcgaacccctgt
ggtgcctgcccgcagagccgggcataaaggcgagcaccaccccgaaccagtccaatttgcttctgcattcactacca
actttacatccacacatcgtactaccacacctgccagtcgggtttgatttctattgaaaggtgcgggggggttggcgc
actgcgtgggttgtgcagccggccgcccggctgtaccagcgatcaggtagcttgggctgtatcttcaagcattacct
35 **tgtctgggcgtaggtttgc****tctaga****ATG****gccgcgtccgtccactgcacccctgatgtccgtggtctgcaacaacaag**
aaccactccgcccgcgccaagctgccaactcctccctgctgccggcttcgacgtggtggtccaggccgcccacc
cgcttcaagaaggagacgacgaccacccgcgccacgtgacgttcgacccccacgaccaactccgagcgcgcc
aagcagcgcaagcacaccatcgacccctcctccccgacttcagcccatccctccttcgaggagtgttcccaag
tccacgaaggagcacaaggaggtggtgcacgaggagtcggccacgtcctgaagggtgcccttcgccgctgcac
40 **ctgtccggcgggcgagccgccttcgacaactacgacacgtccggccccagaaacgtcaacgcccacatcggcctgg**

cgaagctgcgcaaggagtgatcgaccgccgcgagaagctgggcacgccccgctacacgcagatgtactacgcg
 aagcagggcatcatcacggaggagatgctgtactgcgcgacgcgcgagaagctggacccccgagttcgtccgctcc
 gaggtcgcgcgggggccgcgccatcatccctccaacaagaagcacctggagctggagcccatgatcgtgggcccgc
 aagttcttgggtgaaggtaaacgcgaacatcggcaactccgcctggcctcctccatcgaggaggaggtctacaag
 5 gtgcagtggggccaccatgtggggcgccgacaccatcatggacctgtccacggggccgacatccacgagacgcgc
 gagtggatcctgcgaactccgcggtccccgtgggcaccgtccccatctaccaggcgctggagaagggtggacggca
 tcgcggagaacctgaactgggaggtgtccgcgagacgctgatcgagcaggccgagcagggcggtggactacttca
 cgatccacgcggggtgtgctgctgacatccctgaccgccaagcgctgacgggcatcgtgtcccgcggcggc
 tccatccacgcgaagtgtgctgctgctaccacaaggagaacttcgctacgagcactgggacgacatcctggaca
 10 tctgcaaccagtagcagctgcctgtccatcggcgacggcctgcgccccggctccatctacgacgccaacgacacg
 gcccagttcgcgagctgctgaccagggcgagctgacgcgcgcgctgggagaaggacgtgcaggtgatgaa
 cgagggccccggccacgtgcccgtgcacaagatccccgagaacatgcagaagcagctggagtggtgcaacgagg
 cgcccttctacaccctgggccccctgacgaccgacatcgcgcccggtacgaccacatcacctccgcatcggcgcgg
 ccaacatcggcgcctgggcaccgcccgtgtgtgctacgtgacgccaaggagcacctgggctgccaaccgcga
 15 cgacgtgaaggcggtgcatcgctacaagatcgccgcccacgcggccgacctggccaagcagacccccacgc
 ccaggcggtgggacgacgcgctgtccaaggcgcttcgagttccgctggatggaccagttcgcgctgtccctggacc
 ccatgacggcgatgtccttcacgacgagacgctgcccgcggacggcggaaggtcgcccacttctgctccatgtgc
 ggcccaagtctgctccatgaagatcacggaggacatccgcaagtacgccgaggagaacggctacggctccgcc
 gagggaggccatccgcccaggcatggacgcatgtccgaggagttaacatcgccaagaagacgatctccggcga
 20 gcagcacggcgaggtcggcggcgagatctacctgcccagtcctacgtcaaggccgcgcagaagTGAtacgtaa
 cagacgaccttggcaggcgctcggttagggaggtgggtgatggcgctcgcgatgccatcgacgcatccaacgaccgta
 tacgcatcgtccaatgaccgtcggtgtcctctctgctccgtttgtgagatgtctcaggcttgggtgcatcctcggtggcca
 gccacgttgcgcgtcgtgctgtcctctcttgcgcctctgtggtactggaaaatatcatcgaggcccggttttttgcctcc
 atttcttccgctacatcttgaaagcaaacgacaaacgaagcagcaagcaagagcacgaggacggtgaacaagtct
 25 gtcacctgtatacatctatttccccgcgggtgcacctactctctcctgccccggcagagtcagctgccttacgtgacgga
 tcccgcgtctcgaacagagcgcgagaggaacgtgaaggtctgcctctgtcgacactcagcgcgcatcacaccaca
 taaccacctgacgaatgcgcttggttcttctgctcattagcgaagcgctccggttcacacacgtgccacgttggcgaggtggc
 aggtgacaatgatcggtggagctgatggtcgaaacgttcacagcctaggctgggtcgggcctcgtgctggcactccctcc
 catgccgacaaccttctgctgtcaccacgaccacgatgcaacgcgacacgaccgggtgggactgatcggttactgca
 30 cctgcatgcaattgtcacaagcgcatctccaatcgatccgtttgatttctgtgaaaactcgtcgaccgcccgcgtccc
 gcaggcagcgatgacgtgtgctgacctgggtgttctgctgaaaggccagcaaccccaaatcgaggcgatccggaga
 ttgggatctgatccgagcttgaccagatccccacgatcgggcacgggaactgcatcgactcggcgcggaacccagct
 ttcgtaaatgccagattggtgtccgataccttgatttgcatcagcgaacaagacttcagcagcgagcgtatttggcgg
 gcgtgtaccagggtgcatacattgccatttctgctgaccgcttaccggcgagagggtgagttgatggggttggc
 35 aggcacgaaacgcgcgtgcatggtgtgtgtgtgtgttttcggctgcacaattcaatagtcggatgggagcaggtagaa
 ttgggtgttgcgtcgcgtgcatgcctcgccccgtcggtgtcatgaccgggactggaatccccctcgcgacctctctgc
 taacgctcccgactctccgcccgcgcgaggatagactctagttaaccaatcgacaactagtagtATGacgggcgagg
 agatggaggagcgcaaggccaccggctaccgcgagtttccggccgacaggttccccccaacacatgcacgc
 cctgctggccatgggcatctggctgggcgcatccacttaacgcctgtgctgctgttctccttctgttctgcctt
 40 ctccaagttctggtggtgttcggctgctgctgctgttcatgatcctgcccactcgaacctactccaagttcgccgccc
 gcctgtcccgtacatctccaagcagcctgctcctacttccccatcacctgcacgtggaggacatccacgccttcca
 ccccgaccgcgctacgtgttcggcttcgagccccactccgtgctgcccacggcggtggcctggccgacctgac
 cggcttcatgccccgccaagatcaagggtgctggcctcctccgctgttctacaccccttctgcgccacatctgga
 cctggctgggctgacccccgccaccaagaagaacttctcctcctgctggacgcccgtactcctgcatcctggtgcc
 45 cggcgcgctgcaggagaccttccacatggagcccggctccgagatcgcttctgctgccccgcggcgttctgctgc

128

atctcgggcacaaggcgtcgtcgacgtgacgtgccgtgatgagagcaataccgcgctcaaagccgacgcatggcctt
 tactccgcactccaaacgactgtcgtcgtatcttttcggatatctatcttttaagagcgagcacagcgccgggcatgggc
 ctgaaaggcctcgcggccgtgctcgtgggtgggggcccgcgagcgcggtggggcatcgcggcagtgaccaggcgagac
 ggaggaacgcatgggtgagtcgcatcacaaatgcatgtcttgtgtctgtactataatgctagagcatcaccaggggc
 5 ttagtcatcgacactgctttggtcattacagaaattgcacaagggcgctcctccgggatgaggagatgtaccagctcaag
 ctggagcggccttcgagccaagcaggagcgcggcgcatgacgacctaccacatgcgaagagc

[0301] The sequence of the pSZ6377 transforming DNA is provided below.

Relevant restriction sites in the construct are indicated in lowercase, bold and underlined text, and are 5'-3' *BspQI*, *KpnI*, *XbaI*, *SnaBI*, *BamHI*, *AvrII*, *SpeI*, *AscI*,
 10 *ClaI*, *SacI* and *BspQ I*, respectively. *BspQI* sites delimit the 5' and 3' ends of the transforming DNA. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent *FAD2-1* 5' genomic DNA that permits targeted integration at the *FAD2-1* locus via homologous recombination. The *P. moriformis* *LDH1* promoter driving the expression of the *Arabidopsis thaliana* *THIC* gene is indicated by boxed text. The
 15 initiator ATG and terminator TGA for *AtTHIC* are indicated by uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* *HSP90* 3' UTR is indicated by lowercase underlined text. A spacer sequence is represented by lowercase text. The *P. moriformis* *SAD2-1* promoter, indicated by boxed italicized text, is utilized to drive the expression of the *G. mangostana* *FATA1* gene. The
 20 initiator ATG of the sequence encoding the *C. protothecoides* *SAD1* transit peptide (*CpSAD1tp*) is indicated by uppercase, bold italics, and the remainder of the *CpSAD1tp* sequence located between the ATG and the *AscI* site is indicated with lowercase, underlined italics. The *GarmFATA1(G108A)* coding region is indicated by lowercase italics. A sequence encoding a 3X FLAG tag fused to the C-terminus of
 25 *GarmFATA1(G108A)* is represented by uppercase italics, and the TGA terminator codon is indicated with uppercase, bold italics. The *P. moriformis* *SAD2-1* 3' UTR is indicated by lowercase underlined text. The *FAD2-1* 3' genomic region is indicated by bold, lowercase text.

[0302] SEQ ID NO:130 Nucleotide sequence of transforming DNA contained in
 30 pSZ6377

gctcttcggaaggctcattttccagaacaacgaccatggcttgtcttagcgatcgctcgaatgactgctagtgagtcgta
cgctcgaccagtcgctcgcaggagaacgcggcaactgccgagcttcggcttgccagtcgtgactcgtatgtgatcagg
aatcattggcattggtagcattataattcggcttcgcgctgtttatgggcatggcaatgtctcatgcagtcgaccttagt
caaccaattctgggtggccagctccgggcgaccgggctccgtgtcgcgggcaccacctcctgcatgagtaacagggc
 35 **cgccctctctcccacgttggccaactgaataccgtgtcttggggccctacatgatgggctgccttagtcgggctgggacg**
cgcaactgccgcgcaatctgggacgtggtctgaatctccaggcgggtttcccgagaaagaaaggggtccgatttca

aagcagagccatgtgccggccctgtggcctgtgttggcgctatgtagtcacccccctcacccaattgtcgccagttt
 gcgcaatccataaactcaaaactgcagcttctgagctgcgtgttcaagaacacctctggggttctcaccgcgagg
 tcgacggtacc|tccctccgtctctgcactctggcgccctctccgtctctgtggactgacggacgagagtctggcgccgc
 ttttctatccacaccgccctttccgcatcgaagacaccacccatcgtgccgaggtcttcccaatcaccgccctgtggt
 5 cctctctccagccgtgttggctgctgctccacattttccattcgtgccccacgatcctcgccatcttggcgcttgat
 aggcaccctttttcagcacgccctggtgtgtagcacaacctgacctctctaccgcatcgctccctccacacctcagt
 tgactccctcgtcgacgttgacccgcaagctccccatttcctctattgacaatcgcacactgtacatgtatgtcatta
 ttttgcaaaaaaacaggggtcggttctactcctggcagacgacggtgtgctccgcgccgctgaggcggtcgcgga
 cggcaacacccatcgaccgcacgtcgacgagtaacccacctgtcaacgggtgatctcccatcgcgacacccccg
 10 tgaccgtactatgtgctccatacgaacatgaaaaggaccttggctcccgaggcgagctcgtaatcccagggtt
 ggccccgcttccgtggacacccatcgcatcttccggctcgccgctgtcgagcaagcgccctcgtgcgcgaaccttgt
 ggtgctgcccgcagagccgggcataaaggcgagcaccacacccgaaccagtccaatttgccttctgattcactacca
 acttttacatccacacatcgtactaccacacctgcccagtcgggttgatttctattgcaaagggtcgggggggttggcg
 actgctgggttgtgacgcccgcgcccggctgtaccagcgatcaggtagcttgggtgtatcttcaagcattacct
 15 tgtctggcgtaggtttgc|tctaga**ATG**gccgcgtccgtccactgcacctgatgtccgtggtctgcaacaacaag
 aaccactccgcccgcaccaagctgccaaactcctcctgctgccggcttcgacgtggtggtccaggccgcgccacc
 cgcttcaagaaggagacgacgaccacccgcgccacgtgacgttcgacccccacgaccaactccgagcgcgcc
 aagcagcgcaagcacaccatcgacccctcctccccgacttcagcccatccctccttcgaggagtgttcccaag
 tccacgaaggagcacaaggaggtggtgacgaggagtccggccacgtcctgaagggtgcccttccgcccgtgac
 20 ctgtccggcgagcccgccttcgacaactacgacacgtccggccccagaaagctcaacgcccacatcgccctgg
 cgaagctgcgcaaggagtggatcgaccgccgagaagctgggacgccccgtacacgcagatgtactacgcg
 aagcagggcatcatcaggaggagtgtgtactgctgcgacgcgagagaagctggaccccgagtctgtccgctcc
 gaggtcgcgggggccgcccacatcatccctccaacaagaagcacctggagctggagcccatgatcgtgggccc
 aagttcctggtgaaggtaacgcgaacatcggcaactccgcccgtggcctcctccatcgaggaggaggtctacaag
 25 gtgcagtgggccaccatgtggggcgccgacaccatcatggacctgtccacgggcccacatccacgagacgcgc
 gagtggatcctgcgaactccgcggtccccgtgggacccgtcccatctaccaggcgctggagaagggtggacggca
 tcgaggagaacctgaactgggaggtgttccgcgagacgtgatcgagcaggccgagcaggcggtgactacttca
 cgatccacgcgggctgtgctgctgctacatccctgaccgccaagcgctgacgggcatcgtgtcccgcggcgcc
 tccatccacgcgaagtgtgctgctgctaccacaaggagaacttcgctacgagcactgggacgacatcctggaca
 30 tctgcaaccagtagcagctgcctgtccatcggcgacggcctgcgccccggctccatctacgacgccaacgacacg
 gccagttcgccgagctgctgaccagggcgagctgacgcgcccgcgtgggagaaggacgtgcaggtgatgaa
 cgagggccccggccacgtgccatgcacaagatccccgagaacatgcagaagcagctggagtggtgcaacgagg
 cgcccttctacacctgggccccctgacgaccgacatcgcgcccggctacgaccacatcacctccgcatcggcgcgg
 ccaacatcggcgcctgggacccgctgtgtgtacgtgacgccaaggagcacctgggctgccaaccgcga
 35 cgacgtgaaggcggtcatcgctacaagatcgccgccacgcggccgacctggccaagcagacccccacgc
 ccaggcgtgggacgacgcgtgtccaaggcgcttcgagttccgtggatggaccagttcgcgctgtccctggacc
 ccatgacggcgatgtcttccacgacgagacgtgcccgcggacggcggaaggctgccacttctgtccatgtgc
 ggccccaaagtctgtccatgaagatcacggaggacatccgcaagtacgccgaggagaaacggctacggctccgc
 gagggagccatccgccaggcatggacgcatgtccgaggagttaacatcgccaagaagacgatctccggcga
 40 gcagcacggcgaggtcggcggcgagatctacctgcccagtcctacgtcaaggccgcgagaag**TGAtacgtaa**
 cagacgaccttggcaggcgctcggttagggaggtggtggtgatggcgctctcgatgccatcgacgcatccaacgaccgta
 tacgcatcgtccaatgaccgtcggtgtcctctctgctccgttttggtagatgtctcaggcttgggtgcatcctcggtggcca
 gccacgttgcgctgctgcttgcctctcttgcgctctgtggtactggaaaatatcatcgaggccccgtttttgtccc
 atttcttccgctacatcttgaagcaaacgacaaacgaagcagcaagcaaaagacacgaggacggtgaacaagtct
 45 gtcacctgtatacatctattccccgcgggtgcacctactctctcctgccccggcagagtcagctgccttacgtgacgga

[0303] Constructs pSZ6383, pSZ6384 and pSZ6377 were transformed into S8813. Primary transformants were clonally purified and screened under standard lipid
45 production conditions at pH 5. Integration of pSZ6383 or pSZ6384 at the *FAD2-1*

locus was verified by DNA blot analysis. The fatty acid profiles, *sn*-2 profiles and lipid titers of lead strains were assayed in 50-mL shake flasks (Table 23). *FAD2-1* ablation reduced C18:2 to <1% in most strains. Expression of a second copy of *GarmFATA1(G108A)* and *TcDGAT1* (S8990, S8992, S8998 & S8999), or *TcDGAT2* (S8994, S9000 & S9047) elevated C18:0 to >56%. The D5393-28 strain, expressing a second copy of *GarmFATA1(G108A)* without either of the cocoa DGAT genes (pSZ6377) had a similar fatty acid profile, but lower lipid titer. As shown in Table 23, as compared to strain S8813, for strains expressing either *TcDGAT1* or *TcDGAT2*, C16:0 increased from 3.2% to 3.7%-4.0%, C18:0 increased from 45.8% to about 56%, C18:2 decreased from 1.4% to about 1.0%.

[0304] Table 23. Fatty acid profiles of *FAD2-1* ablation strains.

Strain	S8813	D5393-28	S8990	S8992	S8998	S8999	S8994	S9000	S9047
C12:0	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2
C14:0	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C16:0	3.2	3.8	3.7	3.8	3.9	4.0	3.7	3.8	3.5
C16:1 cis-7	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C16:1 cis-9	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C17:0	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.2
C18:0	45.8	56.0	56.6	56.0	56.2	56.0	56.3	56.4	56.5
C18:1	45.9	35.8	35.4	35.9	35.7	35.5	35.9	35.7	35.9
C18:2	1.4	1.0	0.9	1.0	0.9	1.1	0.9	0.9	0.8
C18:3 α	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.3
C20:0	2.0	1.6	1.6	1.5	1.6	1.5	1.5	1.5	1.5
C22:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
C24:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
saturates	52.1	62.6	63.1	62.6	62.9	62.8	62.8	62.9	62.7

[0305] Liquid chromatography and mass spectrometry were used to analyze the TAG composition of final strains. The strains accumulated 68-71% SOS, with trisaturates ranging from 2.5-2.8%. The D5393-28 strain, expressing a second copy of *GarmFATA1(G108A)* without either of the cocoa *DGAT* genes had similar SOS content but slightly higher trisaturates. The TAG composition of a typical Shea stearin and a sample of Kokum butter are shown for comparison

[0306] Table 24. LC/MS TAG profiles of *FAD2-1* ablation strains. La = laurate (C12:0), M = myristate (C14:0), P = palmitate (C16:0), Ma = margarate (C17:0),

S = stearate (C18:0), O = oleate (C18:1), L = linoleate (C18:2), Ln = α -linolenate (C18:3 α), A = arachidate (C20:0), G = (C20:1), B = behenate (C22:0), Lg = lignocerate (C24:0), Hx = hexacosanoate (C26:0). Sat = saturated, U = unsaturated

Strain	D5393-28	S8990	S8992	S8998	S8999	S8994	S9000	S9047	Shea stearin	Kokum butter
OOL									0.4	
LLS									0.2	
POL									0.3	
OOO									1.3	1.7
SOL									1.0	0.4
LaOS + MOP	0.2	0.3	0.3	0.2	0.3	0.3	0.4	0.2		
OOP	0.5	0.2	0.3	0.2	0.2	0.4	0.3	0.2	0.8	0.7
PLS (+ SLnS)	0.6	0.7	0.7	0.7	0.7	0.6	0.6	0.4	0.6	0.3
POP (+ MOS)	1.1	1.0	1.0	1.1	1.1	1.0	1.2	0.8	0.7	0.4
OOS	10.5	10.3	11.3	11.0	11.0	10.9	10.1	10.6	6.4	11.8
SLS (+ PLA)	1.9	1.7	2.0	1.6	2.1	1.8	1.9	1.5	5.5	1.4
POS	8.4	8.5	8.4	8.7	8.9	8.4	10.0	7.7	6.3	4.8
MaOS										0.3
SOG	0.4	0.5	0.5	0.6	0.3	0.5	0.4	0.5		
OOA	0.5	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.2
SOS (+ POA)	68.4	69.7	68.7	69.1	68.3	69.4	68.0	71.4	69.7	76.6
SSP (+ MSA)	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.4	0.2	
SOA + POB	3.9	3.8	3.5	3.6	3.4	3.5	3.5	3.4	4.0	1.0
SSS (+ PSA)	2.6	2.3	2.2	2.1	2.3	2.2	2.3	2.1	2.0	0.5
SOB + LgOP + AOA	0.4	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.4	
SSA (+ PBS)									0.2	
SOLg (+ POHx)									0.3	
SUM (area %)	99.8	99.9	99.8	99.9	99.8	99.9	100.0	99.9	100.0	100.0
Sat-Sat-Sat	3.1	2.8	2.7	2.5	2.7	2.7	2.8	2.5	2.4	0.5
Sat-U-Sat	84.9	85.9	84.7	85.3	85.1	85.0	86.0	85.8	87.5	84.7
Sat-O-Sat	82.4	83.5	82.0	82.9	82.3	82.6	83.4	83.9	81.4	83.1
Sat-L-Sat	2.5	2.4	2.6	2.3	2.8	2.4	2.6	1.9	6.1	1.6
U-U-U/Sat	11.8	11.3	12.4	12.2	12.0	12.2	11.3	11.7	10.6	14.8

EXAMPLE 8 VARIANT *BRASSICA NAPUS* THIOESTERASE

[0307] In this example, we demonstrate the modification of the enzyme specificity of a FATA thioesterase originally isolated from *Brassica napus* (BnOTE, accession CAA52070), by site directed mutagenesis targeting two amino acids positions D124 and D209).

[0308] To determine the impact of each amino acid substitution on the enzyme specificity of the *BnOTE*, the wild-type and the mutant *BnOTE* genes were cloned into a vector enabling expression and expressed in *P. moriformis* strain S8588. Strain S8588 is a strain in which the endogenous FATA1 allele has been disrupted and expresses a *Prototheca moriformis* KASII gene and sucrose invertase. Recombinant strains with FATA1 disruption and co-expression of *P. moriformis* KASII and invertase were previously disclosed in co-owned applications WO2012/106560 and WO2013/15898, herein incorporated by reference.

[0309] Strains that express wild type or mutant *BnOTE* enzymes, constructs pSZ6315, pSZ6316, pSZ6317, or pSZ6318 were expressed in S8588. In these constructs, the *Saccharomyces carlsbergensis* *MEL1* gene (Accession no: AAA34770) was utilized as the selectable marker to introduce the wild-type and mutant *BnOTE* genes into the FAD2-2 locus of *P. moriformis* strain S8588 by homologous recombination using previously described transformation methods (biolistics). The constructs that have been expressed in S8588 are listed in Table 25.

[0310] Table 25. DNA lot# and plasmid ID of DNA constructs that expressing wild-type and mutant BnOTE genes

DNA Lot#	Solazyme Plasmid	Construct
D5309	pSZ6315	FAD2-2::PmHXT1-ScarMEL1-PmPGK:PmSAD2-2 V3-CpSADtp- <i>BnOTE</i> -PmSAD2-1 utr::FAD2-2
D5310	pSZ6316	FAD2-2::PmHXT1-ScarMEL1-PmPGK:PmSAD2-2 V3-CpSADtp- <i>BnOTE</i> (D124A)-PmSAD2-1 utr::FAD2-2
D5311	pSZ6317	FAD2-2::PmHXT1-ScarMEL1-PmPGK:PmSAD2-2 V3-CpSADtp- <i>BnOTE</i> (D209A)-PmSAD2-1 utr::FAD2-2
D5312	pSZ6318	FAD2-2::PmHXT1-ScarMEL1-PmPGK:PmSAD2-2 V3-CpSADtp- <i>BnOTE</i> (D124A, D209A)-PmSAD2-1 utr::FAD2-2

[0311] pSZ6315

The construct pSZ6315 can be written as FAD2-2::PmHXT1-ScarMEL1-PmPGK:PmSAD2-2 V3-CpSADtp-*BnOTE*-PmSAD2-1 utr::FAD2-2. The sequence of the pSZ6315 transforming DNA is provided below. Relevant restriction sites in pSZ6315 are indicated in lowercase, bold and underlining and are 5'-3' *SgrAI*, *Kpn I*, *SnaBI*, *AvrII*, *SpeI*, *AscI*, *ClaI*, *Sac I*, *SbfI*, respectively. *SgrAI* and *SbfI* sites delimit the 5' and 3' ends of the transforming DNA. Bold, lowercase sequences represent FAD2-2 genomic DNA that permit targeted integration at FAD2-2 locus via homologous recombination. Proceeding in the 5' to 3' direction, the *P. moriformis*

HXT1 promoter driving the expression of the *Saccharomyces carlsbergensis* *MEL1* gene is indicated by boxed text. The initiator ATG and terminator TGA for MEL1 gene are indicated by uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PGK 3' UTR is indicated by lowercase underlined text followed by the *P. moriformis* SAD2-2 V3 promoter, indicated by boxed italics text. The Initiator ATG and terminator TGA codons of the wild-type *BnOTE* are indicated by uppercase, bold italics, while the remainder of the coding region is indicated by bold italics in lower case. The three-nucleotide codon corresponding to the target amino acids, D124 and D209, are in lower case, italicized, bolded and wave underlined. The *P. moriformis* SAD2-1 3'UTR is again indicated by lowercase underlined text followed by the FAD2-2 genomic region indicated by bold, lowercase text.

[0312] SEQ ID NO: 131 Nucleotide sequence of transforming DNA contained in pSZ6315

15 caccggcgctgcttcgctgcccgggtgcagcaatcagatccaagtctgacgacttgccgcacgcgccggatccttcaattcca
aagtgtcgtccgctgcttccttcgcttccttgaacatccagcgacgcaagcgagggcgctggggcggtggcgtcccgga
accggcctcggcgacgcgggtgaaattgccgatgtcggcaatgtagtgcgctccgccacctctcaattaagttttcagcgct
20 ggttgggaatgatctgctcatggggcgaagaaggggttcagagggtctttattgttactcgactggcgctaccagcattcgtgc
atgactgattatacatacaaaagtacagctcgttcaatgccctgcgattcctactcccgagcgagcactccttcaccgtcgggtt
gcttcccacgaccacgccggtgaagagggtctgtggcctcgccccctcgagcgcatctttccagccacgtctgtatgattttgcgc
tcatacgtctggcccgtcgaccccaaatgacgggatcctgcataatatcgccgaaatgggatccaggcattcgtcaggaggcgt
cagccccgaggagatgccgtccccgccgattggaagggttagaggggtgaatccccatttcagaaatgggtaccgct
cccgtctggtcctcacgttcgtgtacggcctggatcccggaaagggcggtatgcacgttggtgtgccccgccattggcgccacgtttc
aaagtccccggccagaaatgcacaggaccggcccggtcgcacaggccatgacgaatgccagatttcgacagcaaaacaatctg
25 gaataatcgcaaccattcgcttttgaacgaaacgaaaagacgtgttagcacgtttccgatctgtggggccgaagcatgattg
gggggaggaaagcgtggcccaaggttagccattctgtgccacgcgcgagggaccaatccccggcatcagccttcacgacg
gctgcgccacatataaagccggacgccttccgacacgttcaaacagttttatttctccacttctgaatcaaacaaatcttcaa
ggaagatcctgctcttagcaactcgt**ATG**ttcgcgttctacttctgacggcctgcacatctccctgaagggcggtgttcggcgtctcc
ccctctacaacggcctgggctgacgccccagatgggtgggacaactggaacacgttcgctgcgacgtctccgagcagct
30 gctgctggacacggccgacgcacatctccgacctgggctgaaggacatgggtacaagtacatcatctggacgactgctggt
cctccggccgactccgacggcttctggtcgccgacgagcagaagttcccaacggcatgggccacgtcgccgaccacctgc
acaacaactccttctgttcggcatgtactctccgggcgagtacacgtgcgcccgtaccccggtccctggggccgaggg
aggaggacgcccagttcttcgcaacaaccgctggactacctgaagtacgacaactgtacaacaagggccaggttcggcac
gcccagatctcctaccaccgtacaaggccatgtccgacgcctgaacaagacgggcccctcttactcctgtgcaac
35 tggggccaggacctgaccttactggggtccggcatcgcaactcctggcgatgtccggcgacgtcacggcgaggttcacg
cgccccgactcccgtgcccctgcgacggcgacgagtacgactgaagtacggcggttccactgtccatcatgaacatcctg
aacaagggccgccccatggccagaacgcggggcgtcggggctggaacgacctggacaacctggagggtcggcgtcggcaac
ctgacggacgacgaggagaaggcgacttctcatgtgggcatggtgaagtccccctgatcatcggcgcaacgtgaaca
acctgaaggcctcctactccatctactccaggcgtccgtcatcgccatcaaccaggactccaacggcatccccgccacgcg
40 cgtctggcgctactacgtgtccgacacggacgagtacggccagggcgagatccagatgtggtccggccccctggacaacggc
gaccaggtcgtggcgctgtaacggcggtccgtgtccggccccatgaacacgacctggaggagatcttcttcgactccaac
ctggggtccaagaagctgacctccactgggacatctacgacctgtgggcgaaccgctcgacaactccacggcgctccgcatc
ctggggccgaacaagaccgcccacggcatcctgtacaacgccaccgagcagtcctacaaggacggcctgtccaagaacgaca

cccgcctgttcggccagaagatcggctccctgtcccccacgcgatcctgaacacgaccgtcccccgccacggcatcggttcta
 ccgcctgcgccccctcc**TGA**tacaacttattacgtattctgaccggcgctgatgtggcgcgacgcgctgactctttagactt
 tactcttgaggaaatgaacctttctcgttgctggcatgtaaacattggcgcaattaattgtgtgatgaagaaagggggcacaagat
 ggatcgcgaatgtacgagatcgacaacgatggatgattgttatgaggggccaacctggctcaatcttgcgcattgctcggcgcaatg
 5 tgatccagcggcgctgactctcgaacctggtagtgtgtgcgcaccgggtcgctttagtataaactgatcgattgccatcccgtaact
 cacaagcctactctagctcccattgcgcactcgggcgcccggctcgatcaatgttctgagcggaggcggaagcgctcaggaaatcgct
 tcggcagctggaagcgcatggaatgcggagcggagatcgaatcaggatcccgcgtctcgaacagagcgcgagaggaaacgctga
 aggtctcgcctctgtgcacctcagcggcgatacaccacaataaccacctgacgaatgcgcttggttcttctccattagcgaagcg
 tccggttcacacacgtgccacgttggcgaggtggcaggtgacaatgatcgggtggagctgatggtcgaaacgttcacag**cctagg**gat
 10 atc**gtgaaaactcgctcgaccgcccgctcccgaggcagcgatgacgtgtgctgacctgggtgtttctgcgaagggccagc**
 aaccccaaatcgaggcgatccggagattgggatctgatccgagcttgaccagatccccacgatgcggcagcggaactgc
 atcgactcggcgaggaaaccagctttcgtaaatgccagattggtgtccgataccttgatttgccatcagcgaacaagacttca
 gcagcgagcgtatttggcgggcgctaccaggggtgcatacattgccatttctgtctggaccgcttaccggcgagaggggt
 gagttgatgggggtggcaggcatcgaacgcgcgtgcatggtgtgtgtgtctgttttcggctgcacaatttcaatagtcggatg
 15 ggcgacggtagaattgggtgtgctgcgtgcatgcctcgcccgtcggtgtcatgaccgggactggaatccccctcgcg
 accctctgtaacgctcccgaactctcccgccgcgcaggatagactctagtccaacatcgaca**actagtATG**gccaccg
 catccactttctggcggttaatgccgctgcggcgacctgcgtcgtcggcgggctccgggccccggcgcccagcgaggcccc
 tccccgtgcggggcgcgcctcccagctgcgcaagcccgcctggacccccgcgcgctgatctccgagaccaggggtcc
 atctccccgtgaactctgcacccccgcgacgcctgcgcgcccgcgctgatggaggacggctactctacaaggagaa
 20 gttcatctgctcctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgcaacctgctgcaggaggtggc
 ctgcaaccacgtgcagaagtgcggcttctccaccgacggcttcgccaccacccctgaccatgcgcaagctgcacctgatctggg
 tgaccgcccgcattgcacatcgagatctacaagtacccgcctgggtccgacgtggtggagatcgagacctggtgacagtcgga
 gggccgcatcggcaccgcgcgactggatctgcgcactccgccaccaacgaggtgatcggccgcgcaacctcaagtgg
 gtgatgatgaaccaggacaccgcgcctgcagcgcgtgaccgacgaggtgcgcgacgagtacctggtgtctgccccgc
 25 gagccccgctggccttccccgaggagaaactctcctgaagaagatcccaagctggaggacccccccagctactcca
 tgttggagctgaagccccgcgcgacactggacatgaaccagcacgtgaacaacgtgacctacatcggtgggtgctgg
 agtccatccccaggagatcatcgacaccacgagctgcaggtgatcacctggactaccgcgcgagtgccagcaggacg
 acatcgtggactccctgaccacctccgagatccccgacgacccatctcaagtaccggcaccacggctccgcatgtct
 ccatccagggccacaacgagctccagttctgcacatgtgcgcctgtccgagacggccaggagatcaaccgcccgcac
 30 ccagtggcgcaagaagtctcccgcattgactacaaggaccacgacggcgactacaaggaccacgacatcgactacaagg
 acgacgacgacaag**TGA**atcgatggagcgacgagtgctgcgtgcggggctggcgaggagtgaggacgcctcctcgtctctgtt
 ctgaacggaacaatcgccacccccgcgtacgcgcacgcacatcgagcaacgaagaaaacccccgatgataggtgcgggtgctg
 ccgggatagatccggccgcacatcaaaggccctccgccagagaagaagctccttccagcagactccttctgctgccaaaac
 acttctctgtccacagcaacaccaaaggatgaacagatcaacttgctcctcgctagcttctcggctagcgtgcttgcaacaggtc
 35 cctgcactattatcttctgcttctcctgaattatgcggcagcgagcgtcgtctggcgagcgctccttcgcgcgcccctcgtgat
 cgagtgtacagtcaatgaatggtagctcgcgcctgcgcgaggacgcagaacaacgctgccgcgtgtcttttcacgcgcgact
 ccggcgcttgcgtggtggcaccataaagaacccctcaattctgttttggaagacacggtgtacccccaccacccactgcac
 ctctattattggtattattgacgcgggagtgggcggtgtaccctacaacgtagcttctctagttttagctggctccaccattgtaa
 ttcatgtagaatagtgctggttatgtgagaggtatagtgctgtagcagacggggcgggatgatgtcgtggtggtgatctttg
 40 gctcaaggcgtcgtcagctgacgtgcccgatcatgagagcaataaccgcgtcaaagccgacgcatagccttactccgcaatcca
 aacgactgtcgtcgtatttttggatatctattttaagagcgagcacagcggcgatgggctgaaaggcctcgggccgtgc
 tcgtggtggggccgcgagcgcgtggggcatcgggcagtgaccaggcgacaggggaacgatggtgcgtgcgcaatata
 agatacatgtattgttgcagcagg

[0313] SEQ ID NO: 132 Nucleotide sequence of BnOTE (D124A) in pSZ6316

45 **ATG**gccaccgcatccactttctggcggttaatgccgctgcggcgacctgcgtcgtcggcgggctccgggccccggcgccc
 agcgaggccccctcccgctgcgcgggcgcgcctcccagctgcgcaagcccgcctggacccccgcgcgctgatctccgccc
 accagggctccatctccccgtgaactctgcacccccgcgacgcctgcgcgcccgcgctgatggaggacggctactcct
 acaaggagaagtctatcgtgcgtcctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgcaacctgctgc

aggaggtggcctgcaaccacgtgcagaagtgcggcttctccaccgcccggcttcgccaccaccctgaccatgcgcaagctgca
 cctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctggtccgacgtggtggagatcgagacctgg
 tgccagtccgagggccgcacatcgccacccgcccgcgactggatctctgcgcgactccgccaccaacgaggtgatcgccgcgcca
 cctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagcgcgtgaccgacgaggtgcgcgacgagtacctggtgt
 5 tctgccccgcgagccccgcctggccttccccgaggagaaactcctccctgaagaagatcccaagctggaggaccccgcc
 cagtactccatgctggagctgaagccccgcgcgcccgcactggacatgaaccagcacgtgaacaacgtgacctacatcggt
 ggggtgctggagtccatccccaggagatcatcgacacccacgagctgcaggtgatcacctggactaccgcccgcgagtgcc
 gcaggacgacatcgtggactccctgaccacctccgagatccccgcgaccccatctccaagttcacccggcaccaacggctccg
 ccatgtctccatccagggccacaacgagtcaccagttctgcacatgctgcgcctgtccgagaacggccaggagatcaaccgc
 10 ggccgcacccagtggcgcaagaagtcctcccgcatggactacaaggaccacgacgcgactacaaggaccacgacatcgac
 tacaaggacgacgacgacaagTGA

[0314] The sequence of the pSZ6317 transforming DNA is same as pSZ6315 except
 the D209A point mutation, the *BnOTE* D209A DNA sequence is provided below. The
 three-nucleotide codon corresponding to the target two amino acids, D124 and D209,
 are in lower case, italicized, bolded and wave underlined. pSZ6317 is written as
 15 FAD2-2::PmHXT1-ScarMEL1-PmPGK:PmSAD2-2 V3-CpSADtp-BnOTE (D209A)-
 PmSAD2-1 utr::FAD2-2

[0315] SEQ ID NO: 133 Nucleotide sequence of BnOTE (D209A) in pSZ6317:
 ATGgccaccgcatccacttctcggcgttcaatgcccgtgcggcgacctgcgtcgctcggcgggctccgggccccggcgccc
 20 agcgaggccccctcccgtgcgcgggcgcgcctcccagctgcgcaagccccccctggacccccctgcgcgcgtgatctccgcg
 accagggctccatctccccgtgaactctgcacccccgcgacccgctgcgcgcggcgcgctgatggaggacggctactcct
 acaaggagaagttcatcgtgcgtcctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgccaacctgctgc
 agggaggtggcctgcaaccacgtgcagaagtgcggcttctccaccgacggcttcgccaccaccctgaccatgcgcaagctgca
 cctgatctgggtgaccgcccgcacatcgagatctacaagtaccccgcctggtccgacgtggtggagatcgagacctgg
 25 tgccagtccgagggccgcacatcgccacccgcccgcgactggatctctgcgcgactccgccaccaacgaggtgatcgccgcgcca
 cctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagcgcgtgaccgcccaggtgcgcgacgagtacctggtgt
 tctgccccgcgagccccgcctggccttccccgaggagaaactcctccctgaagaagatcccaagctggaggaccccgcc
 cagtactccatgctggagctgaagccccgcgcgcccgcactggacatgaaccagcacgtgaacaacgtgacctacatcggt
 ggggtgctggagtccatccccaggagatcatcgacacccacgagctgcaggtgatcacctggactaccgcccgcgagtgcc
 30 gcaggacgacatcgtggactccctgaccacctccgagatccccgcgaccccatctccaagttcacccggcaccaacggctccg
 ccatgtctccatccagggccacaacgagtcaccagttctgcacatgctgcgcctgtccgagaacggccaggagatcaaccgc
 ggccgcacccagtggcgcaagaagtcctcccgcatggactacaaggaccacgacgcgactacaaggaccacgacatcgac
 tacaaggacgacgacgacaagTGA

[0316] The sequence of the pSZ6318 transforming DNA is same as pSZ6315 except
 two point mutations, D124A and D209A, the BnOTE (D124A, D209A) DNA
 sequence is provided below. The three-nucleotide codon corresponding to the target
 two amino acids, D124 and D209, are in lower case, italicized, bolded and wave
 underlined. pSZ6318 is written as FAD2-2::PmHXT1-ScarMEL1-
 PmPGK:PmSAD2-2 V3-CpSADtp-BnOTE (D124A, D209A)-PmSAD2-1 utr::FAD2-
 40 2

[0317] SEQ ID NO: 134 Nucleotide sequence of *BnOTE* (D124A, D209A) in pSZ6318

ATGgccaccgcacccactttctcggcggttcaatgcccgctgcggcgacctgcgtcgctcggcgggctccgggccccggcgccc
 agcgaggccccctccccgtgcgcgggacgcctccagctgcgcaagccccctggacccccctgcgcgcctgatctccgccg
 5 accagggctccatctccccgtgaactcctgcacccccgcgacccgctgcgcgcggcgccgctgatggaggacggctactcct
 acaaggagaagttcatcgtgcgtcctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgccaacctgctgc
 aggaggtggcctgcaaccacgtgcagaagtgcggcttctccaccgcccgttcgccaccacacctgaccatgcgcaagctgca
 cctgatctgggtgaccgccccgcagatgcacatcgagatctacaagtacccccgctggtccgacgtggtggagatcgagacctgg
 10 tgccagtccgagggcgcatcgccacccgcgcgactggatcctgcgcgactccgccaccaacgaggtgatcgccgcgcca
 cctccaagtgggtgatgatgaaccaggacacccgcgcgctgcagcgcgtgaccgcccaggtgcgcgacgagtacctggtgt
 tctgccccgcgagccccgctggccttccccgaggagaacaactcctcctgaagaagtcccaagctggaggacccccgc
 cagtactccatgctggagctgaagccccgcgcgacccgactggacatgaaccagcacgtgaacaacgtgacctacatcggt
 ggggtgctggagtccatccccaggagatcatcgacacccacgagctgcaggtgatcacctggactaccgcccgcgagtgcc
 gcaggacgacatcgtggactccctgaccacctccgagatccccgcgaccccatctcaagttcacgggcaccaacggctccg
 15 ccatgtctccatccagggccacaacgagtcacagttcctgcacatgctgcgcctgtccgagaacggccaggagatcaaccgc
 ggccgacccagtgggcgaagaagtctcccgatggactacaaggaccacgacggcgactacaaggaccacgacatcgac
 tacaaggacgacgacgacaagTGA

[0318] The DNA constructs containing the wild-type and mutant *BnOTE* genes were transformed into the parental strain S8588. Primary transformants were clonally purified and grown under standard lipid production conditions at pH5.0. The resulting profiles from representative clones arising from transformations with pSZ6315, pSZ6316, pSZ6317, and pSZ6318 into S8588 are shown in Table 26. The parental strain S8588 produces 5.4% C18:0, when transformed with the DNA cassette expressing wild-type *BnOTE*, the transgenic lines produce ~11% C18:0. The *BnOTE* mutant (D124A) increased the amount of C18:0 by at least 2 fold compared to the wild-type protein. In contrast, the *BnOTE* D209A mutation appears to have no impact on the enzyme activity/specificity of the *BnOTE* thioesterase. Finally, expression of the *BnOTE* (D124A, D209A) resulted in very similar fatty acid profile to what we observed in the transformants from S8588 expressing *BnOTE* (D124A), again indicating that D209A has no significant impact on the enzyme activity.

[0319] Table 26. Fatty acid profiles in S8588 and derivative transgenic lines transformed with wild-type and mutant *BnOTE* genes

Transforming DNA	Sample ID	Fatty Acid Area %			
		C16:0	C18:0	C18:1	C18:2
	pH5; S8588 (parental strain)	3.00	5.43	81.75	6.47
D5309, pSZ6315;	pH5; S8588, D5309-6 ;	3.86	11.68	76.51	5.06

wild-type <i>BnOTE</i>	pH5; S8588, D5309-2 ;	3.50	11.00	77.80	4.95
	pH5; S8588, D5309-9 ;	3.51	10.72	78.03	5.00
	pH5; S8588, D5309-10 ;	3.55	10.69	78.06	4.96
	pH5; S8588, D5309-11 ;	3.61	10.69	78.05	4.95
D5310, pSZ6316, <i>BnOTE</i> (D124A)	pH5; S8588, D5310-6 ;	4.27	31.55	55.31	5.30
	pH5; S8588, D5310-1 ;	4.53	30.85	54.71	6.03
	pH5; S8588, D5310-5 ;	5.21	20.75	65.43	5.02
	pH5; S8588, D5310-10 ;	4.99	19.18	67.75	5.00
	pH5; S8588, D5310-2 ;	4.90	18.92	68.17	4.98
D5311, pSZ6317, <i>BnOTE</i> (D209A)	pH5; S8588, D5311-3 ;	3.50	11.90	76.95	4.98
	pH5; S8588, D5311-4 ;	3.63	11.35	77.44	4.94
	pH5; S8588, D5311-14 ;	3.47	11.23	77.68	4.98
	pH5; S8588, D5311-10 ;	3.60	11.20	77.53	5.00
	pH5; S8588, D5311-12 ;	3.53	11.12	77.59	5.09
D5312, pSZ6318, <i>BnOTE</i> (D124A, D209A)	pH5; S8588, D5312-20 ;	4.79	37.97	47.74	6.01
	pH5; S8588, D5312-40 ;	5.97	22.94	62.20	5.11
	pH5; S8588, D5312-39 ;	6.07	22.75	62.24	5.17
	pH5; S8588, D5312-16 ;	5.25	18.81	67.36	5.09
	pH5; S8588, D5312-26 ;	4.93	18.70	68.37	4.96

EXAMPLE 9 VARIANT *GARCINIA MANGOSTANA* THIOESERASE

[0320] In this example, we demonstrate the ability to modify the activity and specificity of a FATA thioesterase originally isolated from *Garcinia*

- 5 *mangostana* (*GmFATA*, accession O04792), using site directed mutagenesis targeting six amino acid positions within the enzyme and various combinations thereof.

Facciotti *et al* (NatBiotech 1999) had previously altered three of the amino acids (G108, S111, V193). The remaining three amino acids targeted are L91, G96, and T156.

- 10 [0321] To test the impact of each mutation on the activity of the *GmFATA*, the wild-type and mutant genes were cloned into a vector enabling expression within the *P. moriformis* strain S3150. Table 27 summarizes the results from a three day lipid profile screen comparing the wild-type *GmFATA* with the 14 mutants. Three *GmFATA* mutants (DNA lot numbers D3998, D4000, D4003) increased the amount
- 15 of C18:0 by at least 1.5 fold compared to the wild-type protein (DNA lot number D3997). D3998 and D4003 were mutations that had been described by Facciotti *et al* (NatBiotech 1999) as substitutions that increased the activity of the *GmFATA*. Strain S3150 expressing the mutations contained in DNA lot number D4000 was based on research at Solazyme which demonstrated this position influenced the activity of the
- 20 FATB thioesterases. All of the constructs were codon optimized to reflect UTEX

1435 codon usage. Non-mutated GmFATA increases the fatty acid content of C18:0 and decreases the fatty acid content of C18:1 and C18:2. As can be seen in Table 27 the G90A mutant GmFATA increases the fatty acid content of C18:0 and decreases the fatty acid content of C18:1 and C18:2 when compared to the wild-type GmFATA.

5 [0322] Table 27

Algal Strain	DNA #	GmFATA	C14:0	C16:0	C18:0	C18:1	C18:2
<i>P. moriformis</i> S3150	S3150		1.63	29.82	3.08	55.95	7.22
	D3997 pSZ5083	Wild-Type <i>GmFATA</i>	1.79	29.28	7.32	52.88	6.21
	D3998 pSZ5084	S111A, V193A	1.84	28.88	11.19	49.08	6.21
	D3999 pSZ5085	S111V, V193A	1.73	29.92	3.23	56.48	6.46
	D4000 pSZ5086	G96A	1.76	30.19	12.66	45.99	6.01
	D4001 pSZ5087	G96T	1.82	30.60	3.58	55.50	6.28
	D4002 pSZ5088	G96V	1.78	29.35	3.45	56.77	6.43
	D4003 pSZ5089	G108A	1.77	29.06	12.31	47.86	6.08
	D4007 pSZ5093	G108V	1.81	28.78	5.71	55.05	6.26
	D4004 pSZ5090	L91F	1.76	29.60	6.97	53.04	6.13
	D4005 pSZ5091	L91K	1.87	28.89	4.38	56.24	6.35
	D4006 pSZ5092	L91S	1.85	28.06	4.81	56.45	6.47
	D4008 pSZ5094	T156F	1.81	28.71	3.65	57.35	6.31
	D4009 pSZ5095	T156A	1.72	29.66	5.44	54.54	6.26
	D4010 pSZ5096	T156K	1.73	29.95	3.17	56.86	6.21

	D4011 pSZ5097	T156V	1.80	29.17	4.97	55.44	6.27
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[0323] Nucleotide sequence of the *GmFATA* wild-type parental gene expression vector is shown below (D3997, pSZ5083). The plasmid pSZ5083 can be written as THI4a::CrTUB2-NeoR-PmPGH:PmSAD2-2Ver3-CpSAD1tp_GarmFATA1_FLAG-CvNR::THI4a. The 5' and 3' homology arms enabling targeted integration into the Thi4 locus are noted with lowercase; the *CrTUB2* promoter is noted in uppercase italic which drives expression of the neomycin selection marker noted with lowercase italic followed by the *PmPGH* 3'UTR terminator highlighted in uppercase. The *PmSAD2-1* promoter (noted in bold text) drives the expression of the *GmFATA* gene (noted with lowercase bold text) and is terminated with the *CvNR* 3'UTR noted in underlined, lower case bold. Restriction cloning sites and spacer DNA fragments are noted as underlined, uppercase plain lettering. The nucleotide sequence for all of the *GmFATA* constructs disclosed in this example is identical to that of pSZ5083 with the exception of the encoded *GmFATA*. The promoter, 3'UTR, selection marker and targeting arms are the same as described for pSZ5083. The individual *GmFATA* mutant sequences are shown below. The amino acid sequence of the unmutagenized *GmFATA* is shown in Figure 1. The amino acid sequences of the altered *GmFATA* proteins are shown below.

[0324] SEQ ID NO:135 pSZ5083

ccctcaactgcgacgctgggaaccttctccgggcaggcgatgtgcggtgggtttgcct
ccttggcacggctctacaccgctcgagtacgccatgaggcgggtgatggctgtgtcggt
tgccacttcgtccagagacggcaagtcgtccatcctctgctgtgtgtggcgcgacgct
gcagcagtcacctctgcagcagatgagcgtgactttggccatttcacgcactcgagtg
tacacaatccatttttcttaaagcaaatgactgctgattgaccagatactgtaacgc
25 tgatttcgctccagatcgacacagatagcgaccatgttgctgctgctgaaaatctgga
ttccgaattcgaccctggcgctccatccatgcaacagatggcgacacttggtacaat
tctgtcacccatcggcattggagcaggtccacttagattcccgatcacccacgcaca
tctcgctaatagtcattcgttctgtcttccgatcaatctcaagtgagtgtgcatgga
tcttggttgacgatgcggtatgggtttgcgccgctggctgcagggctctgcccaaggc
30 aagctaaccagctcctctccccgacaatactctcgcaggcaaagccgggtcacttgc
cttcagattgccaataaactcaattatggcctctgtcatgccatccatgggtctga
tgaatggtcacgctcgtgtcctgaccgttccccagcctctggcgctccccctgccccgc
ccaccagcccacgcccgcgaggcagtcgctgccaaggctgtctcggaGGTACCCCTTC
TTGCGCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCG
35 CTGCATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGG
CGCTCCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAA
AGACATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCT

ACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTC
 GTTTCAGTCACAACCCGCAAACCTCTAGAATATCAatgatcgagcaggacggcctcca
 cgccggctcccccgccgctgggtggagcgctgttcggctacgactgggcccagca
 gaccatcggctgctccgacgcccgcgtgttccgcctgtccgcccagggccgccccgt
 5 gctgttcgtgaagaccgacctgtccggcgccctgaacgagctgcaggacgaggccgc
 ccgcctgtcctggctggccaccaccggcgctgccctgcgcccgcgtgctggacgtggt
 gaccgaggccggccgagctggctgctgctgggagaggtgcccgccaggacctgct
 gtcctcccacctggcccccgccgagaaggtgtccatcatggccgacgccatgcgccc
 cctgcacaccctggacccccgccacctgccccttcgaccaccaggccaagcaccgcat
 10 cgagcgcgccccgcacccgcatggaggccggcctggtggaccaggacgacctggacga
 ggagcaccaggccctggcccccgccgagctgttcgcccgcctgaaggcccgcatgcc
 cgacggcgaggacctggtggtgacccacggcgacgcctgcctgcccacatcatggt
 ggagaacggccgcttctccggcctcatcgactgcggccgcctgggctggccgaccg
 ctaccaggacatcgccctggccaccgcgacatcgccgaggagctgggcgccgagtg
 15 ggccgaccgcttctggtgctgtacggcatcgccgcccccgactcccagcgcatcgc
 cttctaccgcctgctggacgagttcttctgaCAATTGACGCCCCGCGCGGCACCTG
 ACCTGTTCTCTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCG
 TGCATGATCGTCTCTGGCGCCCCGCGCGCGGTTTGTGCCCCCTCGCGGGCGCCGCGG
 CCGCGGGGGCGCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGC
 20 AGGAAGGCGTTGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAAC
 TAACAACAAAGGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCC
 GTGTAGAGGCTTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTT
 ACGACGTGGTAAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGG
 AGACGATCGGAAGCATCACAGCACAGGATCCCGCGTCTCGAACAGAGCGCGCAGAGG
 25 AACGCTGAAGGTCTCGCCTCTGTGCGACCTCAGCGCGGCATACACCACAATAACCAC
 CTGACGAATGCGCTTGGTTCTTTCGTCCATTAGCGAAGCGTCCGGTTCACACACGTGC
 CACGTTGGCGAGGTGGCAGGTGACAATGATCGGTGGAGCTGATGGTCGAAACGTTCA
 CAGCCTAGGGATATCGTGAAAACCTCGCTCGACCGCCCCGCGTCCCGCAGGCAGCGATG
 ACGTGTGCGTGACCTGGGTGTTTCGTGCGAAAGGCCAGCAACCCCAAATCGCAGGCCA
 30 TCCGGAGATTGGGATCTGATCCGAGCTTGGACCAGATCCCCACGATGCGGCACGGG
 AACTGCATCGACTCGGCGCGGAACCCAGCTTTCGTAAATGCCAGATTGGTGTCCGAT
 ACCTTGATTTGCCATCAGCGAAACAAGACTTCAGCAGCGAGCGTATTTGGCGGGCGT
 GCTACCAGGGTTGCATACATTGCCCATTTCTGTCTGGACCGCTTTACCGGCGCAGAG
 GGTGAGTTGATGGGGTTGGCAGGCATCGAAACGCGCGTGCATGGTGTGTGTCTGT
 35 TTTCGGCTGCACAATTTCAATAGTCGGATGGGCGACGGTAGAATTGGGTGTTGCGCT
 CGCGTGCATGCCCTCGCCCCGTCGGGTGTATGACCGGGACTGGAATCCCCCTCGCG
 ACCCTCCTGCTAACGCTCCCGACTCTCCCGCCCCGCGCGCAGGATAGACTCTAGTTCA
 ACCAATCGACAACCTAGTatggccaccgcatccacttctcggcggttcaatgcccgt
 gcggcgacctgcgtcgctcggcgggctccgggccccggcgcccagcgaggccctcc
 40 ccgtgcgcggggcgcgccatccccccccgcatacgtgggtgtcctcctcctcctcca
 aggtgaaccccctgaagaccgaggccgtggtgtcctccggcctggccgaccgcctgc
 gcctgggctccctgaccgaggacggcctgtcctacaaggagaagttcatcgctgcgt
 gctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgccaacctgctgc
 aggaggtgggctgcaaccacgcccagtcctgtgggctactccaccggcggttctcca
 45 ccacccccaccatgcgcaagctgcgcctgatctgggtgaccgcccgcctgcacatcg
 agatctacaagtaacccgcctggtccgacgtggtggagatcgagtcctggggccagg
 gcgaggggaagatcggcacccgcccgcgactggatcctgcgcgactacgccaccggcc
 aggtgatcgggccgcccacctccaagtgggtgatgatgaaccaggacacccgcccgc
 tgcagaaggtggacgtggacgtgcgcgacgagtacctggtgcactgcccccgcgagc
 50 tgcgcctggccttccccgaggagaacaactcctccctgaagaagatctccaagctgg

5 aggaaccctcccagttactccaagctgggcctggtgccccgcgcgcgcgacctggaca
 tgaaccagcacgtgaacaacgtgacctacatcggtggtgctggagtccatgcccc
 aggagatcatcgacacccacgagctgcagaccatcacccctggactaccgcccgcgagt
 gccagcacgacgacgtggtggactccctgacctcccccgagccctccgaggacgccc
 10 aggcggtgttcaaccacaacggcaccaacgggtccgccaacgtgtccgccaacgacc
 acggctgccgcaacttctgcacctgctgcgctgtccggcaacggcctggagatca
 accgcggccgcaccgagtggtgcaagaagcccacccgcctggtactacaaggaccacg
 acggcgactacaaggaccacgacatcgactacaaggacgacgacgacaagtgaATCG
 ATgcagcagcagctcggatagtagtgcacacactctggacgctggtcgtgtgatggac
 15 tgttgccgcccacacttgccttgacctgtgaatatccctgccgcttttatcaaac
 agcctcagtgtgtttgatcttgtgtgtacgcgcttttgcgagttgctagctgctgtg
 gctatttgcgaataaccacccccagcatccccttccctcggtttcataatcgcttgcatc
 ccaaccgcaacttatctacgctgtcctgctatcccctcagcgctgctcctgctcctgc
 tcactgcccctcgacacagccttgggttgggctccgctgtattctcctggtactgca
 20 acctgtaaaccagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaa
 atggaAAGCTTGAGCTCcagcgccatgccacgccctttgatggcttcaagtacgatt
 acgggtgttggtgtgtgtttgttgcgtagtgtgcatgggttagaataatacacttg
 atttcttgcacggcaatctcggcttgcgcaggttcaaccccatcttcggagtct
 caggtcagccgcgcaatgaccagccgctacttcaaggacttgcacgacaacgcccag
 25 gtgagctatgtttaggacttgattggaaattgtcgtcgacgcataatcgcgctccgc
 gacagcacccaagcaaaatgtcaagtgcgttccgatttgcgtccgcaggtcgatgtt
 gtgatcgtcggcgccggtatccgcccgtctgtcctgcgcttacgagctgaccaagcac
 cctgacgtccgggtacgcgagctgagattcgatttagacataaattgaagattaaacc
 cgtagaaaaatttgatggtcgcgaaactgtgctcgattgcaagaaattgatcgctct
 30 ccactccgcaggtcgccatcatcgagcagggcggttgcctccggcgccgcccgtggc
 tggggggacagctgttctcggccatgtgtgtacgtagaaggatgaatttcagctgggt
 tttcggttcacagctgtttgtgcatgatttgtttcagactattgttgaaatgttttta
 gatttcttaggatgcatgatttgtctgcatgagact

30 **[0325] SEQ ID NO: 136 Amino acid sequence of Gm FATA wild-type parental gene; D3997, pSZ5083. The algal transit peptide is underlined and the FLAG epitope tag is uppercase bold**

35 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGIVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVDSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

40 **[0326] SEQ ID NO:137 Amino acid sequence of Gm FATA S111A, V193A mutant gene; D3998, pSZ5084. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the S111A, V193A residues are lower-case bold.**

45 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFaTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DaDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGIVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVDSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

[0327] SEQ ID NO:138 Amino acid sequence of Gm FATA S111V, V193A mutant gene; D3999, pSZ5085. The algal transit peptide is underlined, the FLAG

epitope tag is uppercase bold and the S111V, V193A residues are lower-case bold.

5 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGF**V**TTPTMRKLRLI
WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
D**a**DVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

10 **[0328] SEQ ID NO: 139 Amino acid sequence of *Gm* FATA G96A mutant gene;
D4000, pSZ5086. The algal transit peptide is underlined, the FLAG epitope tag is
uppercase bold and the G96A residue is lower-case bold.**

15 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEV**a**CNHAQSVGYSTGGFSTTPTMRKLRLI
WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

20 **[0329] SEQ ID NO: 140 Amino acid sequence of *Gm* FATA G96T mutant gene;
D4001, pSZ5087. The algal transit peptide is underlined, the FLAG epitope tag is
uppercase bold and the G96T residue is lower-case bold.**

25 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEV**t**CNHAQSVGYSTGGFSTTPTMRKLRLI
WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

**[0330] SEQ ID NO: 141 Amino acid sequence of *Gm* FATA G96V mutant gene;
D4002, pSZ5088. The algal transit peptide is underlined, the FLAG epitope tag is
uppercase bold and the G96V residue is lower-case bold.**

30 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEV**v**CNHAQSVGYSTGGFSTTPTMRKLRLI
WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
35 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

**[0331] SEQ ID NO: 142 Amino acid sequence of *Gm* FATA G108A mutant
gene; D4003, pSZ5089. The algal transit peptide is underlined, the FLAG epitope
tag is uppercase bold and the G108A residue is lower-case bold.**

40 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYST**a**GFSTTPTMRKLRLI
WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPSQYSKLGVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

45 **[0332] SEQ ID NO: 143 Amino acid sequence of *Gm* FATA L91F mutant gene;
D4004, pSZ5090. The algal transit peptide is underlined, the FLAG epitope tag is
uppercase bold and the L91F residue is lower-case bold.**

50 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**f**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV

DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQYSLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

- 5 **[0333] SEQ ID NO: 144 Amino acid sequence of *Gm* FATA L91K mutant gene; D4005, pSZ5091. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the L91K residue is lower-case bold**

10 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**k**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQYSLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

- 15 **[0334] SEQ ID NO:145 Figure 10. Amino acid sequence of *Gm* FATA L91S mutant gene; D4006, pSZ5092. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the L91S residue is lower-case bold**

20 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**s**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQYSLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

- 25 **[0335] SEQ ID NO: 146 Amino acid sequence of *Gm* FATA G108V mutant gene; D4007, pSZ5093. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the G108V residue is lower-case bold.**

30 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**LL**LQEVGCNHAQSVGYST**v**GFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQYSLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

- 35 **[0336] SEQ ID NO: 147 Amino acid sequence of *Gm* FATA T156F mutant gene; D4008, pSZ5094. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the T156F residue is lower-case bold.**

40 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**LL**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIG**f**RRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQYSLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

- 45 **[0337] SEQ ID NO: 148 Amino acid sequence of *Gm* FATA T156A mutant gene; D4009, pSZ5095. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the T156A residue is lower-case bold.**

50 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**LL**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIG**a**RRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQYSLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

[0338] SEQ ID NO: 149 Amino acid sequence of *Gm FATA T156K* mutant gene; D4010, pSZ5096. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the T156K residue is lower-case bold.

5 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
WVTARMHIEIYKYPAWSDDVVEIESWGQGEKGIGRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPPESEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

10 **[0339] SEQ ID NO: 150 Amino acid sequence of *Gm FATA T156V* mutant gene; D4011, pSZ5097. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the T156V residue is lower-case bold.**

15 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVSSSSSKVNPLKTEAVVSSGLADR
LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
WVTARMHIEIYKYPAWSDDVVEIESWGQGEKGIGRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPPESEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

[0340] SEQ ID NO: 151 Nucleotide sequence of the *GmFATA S111A*, V193A

20 mutant gene (D3998, pSZ5084). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083.

atggccaccgcacccactttctcggcggttcaatgcccgcctgcggcgacactgcgtcgc
tcggcggggctccggggccccggcgccccagcgaggccctccccgtgcgcgggcgcgc
atccccccccgcacatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
25 accgagggcgtggtgtcctcgggcctggccgaccgcctgcgcctgggctccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgcgtgctacgaggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
cacgcccagtcctgtgggctactccaccggcggttcgccaccacccccaccatgcgc
aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
30 gcctggtccgacgtggtggagatcgagtcctggggccaggggcgagggaagatcggc
accgcccgcgactggatcctgcgcgactacgccaccggccaggatgacggccgcgc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgcg
gacgtgcgcgacgagtagctggtgactgccccgcgagctgcgcctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
35 tccaagctgggcctggtgccccgcccgcgcgcgacctggacatgaaccagcacgtgaac
aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
40 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcgccgcaccgag
tggcgcaagaagcccaccgcacatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

[0341] SEQ ID NO: 152 Nucleotide sequence of the *GmFATA S111V*, V193A

45 mutant gene (D3999, pSZ5085). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083.

atggccaccgcatccacttttctcggcggttcaatgcccgtgcgggcgacctgcgtcgc
 tcggcggggtccggggccccggcgcccagcgaggccccctccccgtgcgcgggcgcgcc
 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
 accgagggcgtggtgtcctccggcctggccgaccgctgcgccctgggctccctgacc
 5 gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtcctgtgggtactccaccggcggttctgtcaccacccccaccatgcgc
 aagctgcgccctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggcaagatcggc
 10 acccgccgcgactggatcctgcgcgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccggcgccctgcagaaggtggacgcg
 gacgtgcgcgacgagtacctggtgcactgcccccgcgagctgcgccctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
 tccaagctgggcctggtgccccgcccgcgcccagcctggacatgaaccagcacgtgaac
 15 aacgtgacctacatcggttgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcaccttgactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgccctgtccgggaacggcctggagatcaaccggcgcccgacccgag
 20 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 cagcacatcgactacaaggacgacgacgacgacaagtga

[0342] SEQ ID NO: 153 Nucleotide sequence of the *GmFATA* G96A mutant gene
 (D4000, pSZ5086). The promoter, 3'UTR, selection marker and targeting arms are
 the same as pSZ5083

25 atggccaccgcatccacttttctcggcggttcaatgcccgtgcgggcgacctgcgtcgc
 tcggcggggtccggggccccggcgcccagcgaggccccctccccgtgcgcgggcgcgcc
 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
 accgagggcgtggtgtcctccggcctggccgaccgctgcgccctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgaggtgggcatc
 30 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtggcgtgcaac
 cagccccagtcctgtgggtactccaccggcggttctccaccacccccaccatgcgc
 aagctgcgccctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggcaagatcggc
 acccgccgcgactggatcctgcgcgactacgccaccggccaggtgatcgcccgcgcc
 35 acctccaagtgggtgatgatgaaccaggacacccggcgccctgcagaaggtggacgtg
 gacgtgcgcgacgagtacctggtgcactgcccccgcgagctgcgccctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
 tccaagctgggcctggtgccccgcccgcgcccagcctggacatgaaccagcacgtgaac
 aacgtgacctacatcggttgggtgctggagtccatgccccaggagatcatcgacacc
 40 cagagctgcagaccatcaccttgactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgccctgtccgggaacggcctggagatcaaccggcgcccgacccgag
 45 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 cagcacatcgactacaaggacgacgacgacgacaagtga

[0343] SEQ ID NO: 154 Nucleotide sequence of the *GmFATA* G96T mutant gene (D4001, pSZ5087). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083

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5  atggccaccgcatccacttttctcggcggttcaatgcccgctgcggcgacctgcgtcgc
   tcggcggggctccgggccccggcgcccagcgaggccctccccgtgcgcggggcgcgcc
   atccccccccgcatcatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
   accgaggccgtggtgtcctccggcctggccgaccgctgcgcctgggctccctgacc
   gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
   aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgacgtgcaac
10  cagccccagtccgtgggctactccaccggcggttctccaccacccccaccatgcgc
   aagctgcgcctgatctgggtgaccgcccgcacatgcacatcgagatctacaagtacccc
   gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggaagatcggc
   acccgccgcgactggatcctgcgcgactacgccaccggccaggtgatcgcccgcgcc
   acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
15  gacgtgcgcgacgagtacctggtgcactgcccccgcgagctgcgcctggccttcccc
   gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
   tccaagctgggcctggtgccccgcgcgcccagcctggacatgaaccagcacgtgaac
   aacgtgacctacatcggctgggtgctggagtcctatgccccaggagatcatcgacacc
   cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
20  gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
   aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
   ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcgcccgacccgag
   tggcgcaagaagcccaccgcacatggactacaaggaccacgacggcgactacaaggac
   cagacatcgactacaaggacgacgacgacaagtga

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[0344] SEQ ID NO: 155 Nucleotide sequence of the *GmFATA* G96V mutant gene (D4002, pSZ5088). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083.

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30  atggccaccgcatccacttttctcggcggttcaatgcccgctgcggcgacctgcgtcgc
   tcggcggggctccgggccccggcgcccagcgaggccctccccgtgcgcggggcgcgcc
   atccccccccgcatcatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
   accgaggccgtggtgtcctccggcctggccgaccgctgcgcctgggctccctgacc
   gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
   aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtggtgtgcaac
   cagccccagtccgtgggctactccaccggcggttctccaccacccccaccatgcgc
35  aagctgcgcctgatctgggtgaccgcccgcacatgcacatcgagatctacaagtacccc
   gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggaagatcggc
   acccgccgcgactggatcctgcgcgactacgccaccggccaggtgatcgcccgcgcc
   acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
   gacgtgcgcgacgagtacctggtgcactgcccccgcgagctgcgcctggccttcccc
40  gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
   tccaagctgggcctggtgccccgcgcgcccagcctggacatgaaccagcacgtgaac
   aacgtgacctacatcggctgggtgctggagtcctatgccccaggagatcatcgacacc
   cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
   gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
45  aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc

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ctgcacctgctgcgccgtgtccggcaacggcctggagatcaaccgcgcccgccaccgag
tggcgcaagaagcccacccgcacatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

[0345] SEQ ID NO: 156 Nucleotide sequence of the *GmFATA* G108A mutant gene

- 5 (D4003, pSZ5089). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ50836.

atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcgccgacctgcgtcgc
tcggcggggtctccgggccccggcgcccgagcgaggccctccccgtgctgcgggcgcgcc
atccccccccgcacatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
10 accgagggcgtggtgtcctccggcctggccgaccgctgctgctgggctccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
cacgcccagtcctggtgggtactccaccgcccgttctccaccacccccaccatgcgc
aagctgctgctgatctgggtgaccgcccgcacatgcacatcgagatctacaagtacccc
15 gctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
accgcccgcgactggatcctgctgctgactacgccaccggccaggtgatcgcccgcgcc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
gacgtgctgctgacgagtagctggtgactgcccccgagctgctgctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
20 tccaagctgggctggtgccccgcccgcgacacctggacatgaaccagcacgtgaac
aacgtgacctacatcggctgggtgctggagtcacatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctcccccgagccctccgaggacgcccaggcgctgttcaaccac
aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
25 ctgcacctgctgctgctgtccggcaacggcctggagatcaaccgcgcccgccaccgag
tggcgcaagaagcccacccgcacatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

[0346] SEQ ID NO: 157 Nucleotide sequence of the *GmFATA* L91F mutant gene

- (D4004, pSZ5090). The promoter, 3'UTR, selection marker and targeting arms are
30 the same as pSZ5083

atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcgccgacctgcgtcgc
tcggcggggtctccgggccccggcgcccgagcgaggccctccccgtgctgcgggcgcgcc
atccccccccgcacatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
35 accgagggcgtggtgtcctccggcctggccgaccgctgctgctgggctccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacttctgagaggtgggctgcaac
cacgcccagtcctggtgggtactccaccgcccgttctccaccacccccaccatgcgc
aagctgctgctgatctgggtgaccgcccgcacatgcacatcgagatctacaagtacccc
gctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
40 accgcccgcgactggatcctgctgctgactacgccaccggccaggtgatcgcccgcgcc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
gacgtgctgctgacgagtagctggtgactgcccccgagctgctgctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
tccaagctgggctggtgccccgcccgcgacacctggacatgaaccagcacgtgaac
45 aacgtgacctacatcggctgggtgctggagtcacatgccccaggagatcatcgacacc

cacgagctgcagaccatcacccctggactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgccctgtccgggaacggcctggagatcaaccgcgggccgcaccgag
 5 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 cacgacatcgactacaaggacgacgacgacgacaagtga

[0347] SEQ ID NO: 158 Nucleotide sequence of the *GmFATA* L91K mutant gene
 (D4005, pSZ5091). The promoter, 3'UTR, selection marker and targeting arms are
 the same as pSZ5083.

10 atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcggcgacctgcgtcgc
 tcggcggggtccggggccccggcgcccagcgaggccctccccgtgcgcggggcgcgcc
 atccccccccgcgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgctgcgccctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
 15 aacaagaccgccaccgtggagaccatcgccaacaagctgcaggaggtgggctgcaac
 cacgcccagtcctgtgggctactccaccggcggttctccaccacccccaccatgcgc
 aagctgcgccctgatctgggtgaccgcccgcgcatgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggcaagatcggc
 acccgccgcgactggatcctgcgcgactacgccaccggccagggtgatcgcccgcgcc
 20 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
 gacgtgcgcgacgagtagctggtgcactgcccccgcgagctgcgccctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
 tccaagctgggctggtgccccgcgcgcgacactggacatgaaccagcacgtgaac
 aacgtgacctacatcggtggtggtgctggagtcctatgccccaggagatcatcgacacc
 25 cacgagctgcagaccatcacccctggactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgccctgtccgggaacggcctggagatcaaccgcgggccgcaccgag
 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 30 cacgacatcgactacaaggacgacgacgacgacaagtga

[0348] SEQ ID NO: 159 Nucleotide sequence of the *GmFATA* L91S mutant gene
 (D4006, pSZ5092). The promoter, 3'UTR, selection marker and targeting arms are
 the same as pSZ5083.

35 atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcggcgacctgcgtcgc
 tcggcggggtccggggccccggcgcccagcgaggccctccccgtgcgcggggcgcgcc
 atccccccccgcgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgctgcgccctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaactcgtgacaggaggtgggctgcaac
 40 cacgcccagtcctgtgggctactccaccggcggttctccaccacccccaccatgcgc
 aagctgcgccctgatctgggtgaccgcccgcgcatgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggcaagatcggc
 acccgccgcgactggatcctgcgcgactacgccaccggccagggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
 45 gacgtgcgcgacgagtagctggtgcactgcccccgcgagctgcgccctggccttcccc

gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
 tccaagctgggctggtgccccgcccgcgcgacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 5 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
 cagacatcgactacaaggacgacgacgacaagtga

- 10 **[0349] SEQ ID NO: 160** Nucleotide sequence of the *GmFATA* G108V mutant gene (D4007, pSZ5093). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083.

atggccaccgcatccactttctcggcggttcaatgcccgtgcggcgacctgcgtcgc
 tcggcgggctccgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgcc
 15 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgcctgcgcctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgcgtgtgtacgagggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtccgtgggctactccaccgtcggcttctccaccacccccaccatgcgc
 20 aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccagggcgagggaagatcggc
 accgcccgcgactggatcctgcgcgactacgccaccggccaggatgcggccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
 gacgtgcgcgacgagtacctggtgcactgccccgcgagctgcgcctggccttcccc
 25 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
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 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 30 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
 cagacatcgactacaaggacgacgacgacaagtga

- [0350] SEQ ID NO: 161** Nucleotide sequence of the *GmFATA* T156F mutant gene (D4008, pSZ5094). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083.

atggccaccgcatccactttctcggcggttcaatgcccgtgcggcgacctgcgtcgc
 tcggcgggctccgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgcc
 40 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgcctgcgcctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgcgtgtgtacgagggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtccgtgggctactccaccggcggttctccaccacccccaccatgcgc
 aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 45 gcctggtccgacgtggtggagatcgagtcctggggccagggcgagggaagatcggc

5 ttcgcccgcgactggatcctgcgcgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
 gacgtgcgcgacgagtacctggtgcactgccccgcgagctgcgcctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggaccctcccagtac
 10 tccaagctgggcctggtgccccgcccgcgccgacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcaccttgactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
 15 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcccgcaccgag
 tggcgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaaggac
 cagacatcgactacaaggacgacgacgacaagtga

[0351] SEQ ID NO: 162 Nucleotide sequence of the *GmFATA* T156A mutant gene
 (D4009, pSZ5095). The promoter, 3'UTR, selection marker and targeting arms are
 15 the same as pSZ5083

atggccaccgcataccacttttctcggcggttcaatgcccgcctgcggcgacctgcgtcgc
 tcggcggggtcctcgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgcc
 atccccccccgcatacatcgtggtgtcctcctcctcctccaaggtgaacccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgcctgcgcctgggctccctgacc
 20 gaggacggcctgtcctacaaggagaagttcatcgtgcgcctgctacgagggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtcctggtggtactccaccggcggttctccaccacccccaccatgcgc
 aagctgcgcctgatctgggtgaccgcccgcacatgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggggcgagggaagatcggc
 25 gcgcgcccgcgactggatcctgcgcgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
 gacgtgcgcgacgagtacctggtgcactgccccgcgagctgcgcctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggaccctcccagtac
 tccaagctgggcctggtgccccgcccgcgccgacctggacatgaaccagcacgtgaac
 30 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcaccttgactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcccgcaccgag
 35 tggcgcaagaagcccaccgcatggactacaaggaccacgacggcgactacaaggac
 cagacatcgactacaaggacgacgacgacaagtga

[0352] SEQ ID NO: 163 Nucleotide sequence of the *GmFATA* T156K mutant gene
 (D4010, pSZ5096). The promoter, 3'UTR, selection marker and targeting arms are
 the same as pSZ5083.

40 atggccaccgcataccacttttctcggcggttcaatgcccgcctgcggcgacctgcgtcgc
 tcggcggggtcctcgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgcc
 atccccccccgcatacatcgtggtgtcctcctcctcctccaaggtgaacccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgcctgcgcctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgcgcctgctacgagggtgggcatc
 45 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac

cacgcccagtcctgtgggtactccaccggcggtttctccaccacccccaccatgcgc
 aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccagggcgagggcaagatcggc
 aagcgccgcgactggatcctgcgcgactacgccaccggccaggtgatcggccgcgc
 5 acctccaagtgggtgatgatgaaccaggacacccggcgctgcagaaggtggacgtg
 gacgtgcgcgacgagtacctggtgcactgccccgcgagctgcgcctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
 tccaagctgggcctggtgccccgcgcgacacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 10 cagagctgcagaccatcacctggactaccggcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgagggcgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcggccgcaccgag
 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 15 cagacatcgactacaaggacgacgacgacaagtga

[0353] SEQ ID NO: 164 Nucleotide sequence of the *GmFATA* T156V mutant gene
 (D4011, pSZ5097). The promoter, 3'UTR, selection marker and targeting arms are
 the same as pSZ5083

atggccaccgcatccactttctcggcggttcaatgcccgctgcggcgacctgcgtcgc
 20 tcggcggggtccgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgc
 atcccccccgcatcatcgtggtgtcctcctcctccaaggtgaacccctgaag
 accgagggcgtggtgtcctccggcctggccgaccgctgcgcctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 25 cacgcccagtcctgtgggtactccaccggcggtttctccaccacccccaccatgcgc
 aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccagggcgagggcaagatcggc
 gtgcgcgcgactggatcctgcgcgactacgccaccggccaggtgatcggccgcgc
 acctccaagtgggtgatgatgaaccaggacacccggcgctgcagaaggtggacgtg
 30 gacgtgcgcgacgagtacctggtgcactgccccgcgagctgcgcctggccttcccc
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 tccaagctgggcctggtgccccgcgcgacacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
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 35 gtggactccctgacctccccgagccctccgaggacgccgagggcgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcggccgcaccgag
 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 cagacatcgactacaaggacgacgacgacaagtga

40

SEQUENCES

SEQ ID NO: 1

gcgagggggtc tgcctgggccc agccgctccc tctgaacacg ggacgcgtgg tccaattcgg 60
 gcttcggggac cttttggcgg tttgaacgcc tgggagaggg cgcccgcgag cctggggacc 120
 5 cccggcaacgg cttccccaga gcctgccttg caatctcgcg cgtcctctcc ctgagcacgt 180
 ggcggttcca cgtgtgggtcg ggcgccccgg actagctcac gtcgtgacct agcttaatga 240
 acccagccgg gcctgcagca ccaccttaga ggttttgatt atttgattag accaatctat 300
 tcacc 305

10 SEQ ID NO: 2

ggcgaaataga ttggtataat gaaataatca aaacctctta ggcggtgcta caggccccggc 60
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 ccgccacgtg ctgagggaga ggacgcgcga gattgcaagg caggctctgg ggaagccgtt 180
 gccgggggtcc ccaggctcgc gggcgcccca tccctggcgt tcaaaccgcc aaaggggtccc 240
 15 gaagccccgaa ttggaccacg cgtcccgtgt ttagaggag cggctggccc aggcagaccc 300
 ctgc 305

SEQ ID NO: 3

ggtgaataga ttggtctaata caaataatca aaacctctaa ggtggtgctg caggccccggc 60
 20 tgggttcatt aagctagggtc acgacgtgag ctagtccggg acgcccgacc acacgtggaa 120
 ccgccacgtg ctgagggaga ggacgcgcga gattgcaagg caggctctgg ggaagccgtt 180
 gccgggggtcc ccaggctcgc gggcgccctc tcccaggcgt tcaaaccgcc aaaggggtccc 240
 gaagccccgaa ttggaccacg cgtcccgtgt tcagaggag cggctggccc aggcagaccc 300
 ctgc 305

25

SEQ ID NO: 4

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 ccgatctgtg cccaagcatg tgtcggagct cggctgtctc ggacgcgagc tcctgtgcaa 300
 ggggcttgca tcgagaatgt caggcgatag aactgcacg ttggggacac ggaggtgccc 360
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 gccacaaccg cagcaggcgt tcaccacaaa gaattttcca gatcgtgatg cgcagtgtatc 480
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 cagggcaccc atctgcgtcc cttttcagga accaccacc gctgccaggc accttcgcca 600
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 ggatctgccc gcatacgggtc agtccccagc acaagggaagc caagagtaca ggctgttggt 720
 gtcgatggag gagtggccgt tcccacaagt agtgagcggc agctgctcaa cggcttcccc 780

ctgttcatct tggcaaagcc agtgacttcc tacaagtatg tgatgcagat cggcactgca 840
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 gctgcttggg aggaatcatc ggcacacgcc cgtgccgtgc ccgcgccccg cgcccgctcg 960
 5 gaaaggcccc cggttaggac actgccgcgt cagccagtcg tgggatcgat cggacgtggc
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 gaatcctcgc ccggacaccc tcatcacacc ccacatttcc ctgcaagcaa tcttgccgac
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 aaaatagtca agatccattg ggtttaggga acacgtgcga gactgggcag ctgtatctgt
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 cc
 1322

SEQ ID NO: 5

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 ctgctttgag gccgaggacg gtgcgggacg ctcagatcca tcagcgatcg cccaccctc 180
 agagcacctc cgatccaagg caatactatc aggcaaagt tccaaattca aacattccaa 240
 aatcacgcca gggactggat cacacacgca gatcagcgcc gttttgctct ttgcctacgg 300
 25 gcgactgtgc cacttgctga cccctgggtga cgggaggac cagcctgcg gttggcatcc 360
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 gaggcaaaca catagggaca cctgcgtctt tcaatgcaca gacatctatg gtgcccattg 600
 30 atataaaatg ggctacttct gactcaaacc aacgcaaact gcgctatggc aaggccggcc 660
 aagggttgaa tcccggtctg tctggatttg agtttggtgg ggctatcacg tgacaatccc 720
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 c 841

35

SEQ ID NO: 6

tcggagctaa agcagaaact gaacaagact tgcgttcgca tacttggtgac actgaatagg 60
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 ctgctttgag gccgaggacg gcgcggaacg cacagatcca tcagcgatcg cccaccctc 180
 40 agagtacatc cgatccaagg caatactatc aggcaaagt tccaaattca aacattccaa 240
 aattacgtca gggactggat cacacacgca gatcagcgcc gttttgctct ttgcctacgg 300

gcgactgtgc cacttgtcga cgcttgggtga cgggagggac cacgcctgcg gttggcatcc 360
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 gcacattatg cgttttttgga tgcctgagca gcgggcgtgc aggaatctgg tctcgccaga 480
 ttcaccgaag atgcggccat cggagcgagg cgagggtgtg gtggccacgc caggcagtgt 540
 5 gaggcaaaca cacagggaca tctgcttctt tcgatgcaca gacatctatg ttgcccgtgc 600
 atataaaatg ggctacttct gaatacaaac aacgcaaaact tcgctatggc aaggccggcc 660
 aaggttggaa tcccgggtctg tctggatttg agtttgtggg ggctatcacg tgacaatccc 720
 tgggattggg cggcagcagc gcacggcctg gatggcaatg gcgcactaat actgctgaaa 780
 gcacggctct gcaccccttt ctcttgacct gcgattggtc cttttcgcaa gcgtgatcat 840
 10 c 841

SEQ ID NO: 7

caccgatcac tccgtcgccg cccaagagaa atcaacctcg atggagggcg aggtggatca 60
 gaggtatttg ttatcggtcg ttcttagtct caatcaatcg tacaccttgc agttgcccga 120
 15 gtttctccac acatacagca cctcccgtc ccagcccatt cgagcgacct aatccgggcg 180
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 caggaccaca tccagcccag gatcttcgac tggctcagag ctgacctca cgcgccacag 300
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 ttgactggct acattgtctg tctgtcgcga gtgcgcctgg gcccttacgg tgggacactg 420
 20 gaactccgcc ccgagtcgaa cacctagggc gacgcccga gcttggcatg acagctctcc 480
 ttgtgttcta aataccttgc gcgtgtggga ga 512

SEQ ID NO: 8

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 gcgatcccag cgatcgtcgt cgcttcagtg ctgaccggtg gaaagcagga gatctcgggc 240
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SEQ ID NO: 9

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 ccctgacctt gctgcaaggc tctccgaccg cacgcaaggc gcagccggcg cgtccggagg 180
 cgatcggatc caatccagtc gtcctccgc agcccgggca cgtttgccca tgcaggccct 240
 tccacaccgc tcaagagact cccgaacacc gccactcgg cactcgcttc ggctgcccag 300

tgcgcggtttg agtttgcctt gccacagaag acacc 335

SEQ ID NO: 10

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ccatattctt tttgctggat gcttgctcgt ctcgatgaca attgtgaacc tcttgtgtga 120
ccctgaccct gctgcaaggc tctccgaccg cacgcaaggc gcagccggcg cgtccggagg 180
cgatcggatc caatccagtc gtccctcccg agccccgggca cgtttgccc a tgcaggccct 240
tccacaccgc tcaagagact cccgaacacc gccactcgg cactcgcttc ggctgccgag 300
tgcgcggtttg agtttgcctt gccacaggag acatc 335

10

SEQ ID NO: 11

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agacgtcgcg gagcctcgtc ccaaaggccc tttctgatcg agcttgctgt cacttgagc 120
ctttaagttg cgcgcgcgat gggataaccg agctgatctg cactcagatt ttggtttgtt 180
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agaccgtgtt gccggcattt acccggtcat cgccagcgcg tcgggacgac aaggccttat 300
cctgtgctga gacgctcgcg cacgtttata aaattgtggg taccgcggtg tgcacagcgt 360
tcaacacgcg ccacgccgaa attggttggg gggggagcac gtatgggact gacgtatggc 420
cagcagcgaa cactcaccga acaagtgcc aatgtatacct tgcatacatg atgctccggc 480
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35 SEQ ID NO: 12

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 ggccagcagc gaacactcac caaacagggtg ccaatgtata gcttgcatca atgatgctct 480
 ggcagcttcg attgactgtc tcgaaaaagt gtgtgcaaac agattatgtg gccgctctgt 540
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 taaatggctt acgcattccc ccccgggcgg ctcacggggg gtgtgggtgaa ccctgccagc 840
 10 tgatcaagtg cttgctgacg tcggccaggg aggtgtatgt gatttggccg tggggcgtga 900
 gttatcctac cgccggaccc gcgaagtcac atgacgaatg gccgtgcggg atgacgagag 960
 cagggctcgc tctttcttcg ccggcccggc ttcattggagg acaataataa aggggtggcca 1020
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 15 atccagttca cccgtatatg cacga 1105

SEQ ID NO: 13

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SEQ ID NO: 14

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SEQ ID NO: 15

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10 SEQ ID NO: 16

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SEQ ID NO: 17

Prototheca moriformis (UTEX 1435) Amt02 promoter

20

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SEQ ID NO: 18

35 *Prototheca moriformis* (UTEX 1435) Amt03 promoter

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5

SEQ ID NO: 19 pSZ3840/D2554 transforming construct (*CpauLPAAT1*)

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SEQ ID NO: 20 pS23841/D2555 (CpailPAAT1)

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SEQ ID NO: 21 **pSZ3842/D2556 (CignealPAAT1)**

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SEQ ID NO: 23 CpauLPAAT1

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15

SEQ ID NO: 24 CprocLPAAT1

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SEQ ID NO: 25 CprocLPAAT1b

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 EK SEQ ID NO:

30

SEQ ID NO: 26 CprocLPAAT2a

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 DTGRPIKSLLVISWAVLEVFQAVKFLQWSSLLSSWKGKLAFCGIGLGIITLLMHILILFSQSERSTPAK
 VAPAKAIEGESSKTEMEKEK

SEQ ID NO: 27 CprocLPAAT2b

40 IVNLVQAVCFVLVRPLSKNTYRRINRVVAELLWLLELVWLIDWWAGVKIKVFTDHFTHLMGKEHALVIC
 NHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEARNWAKDESTLKSGLNRLK
 DYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPNRNLIPTKGFVSSVSHMRSFVPAIYDVTVAI
 PKTSPPTLIRMFQKQSSVLHVHLKRHVMDLPESDDAVAQWCRDIFVEKDALLDKHNADDTFSGQELQ
 DTGRPIKSLLV

45

SEQ ID NO: 28 CpailLPAAT1

MAIPSAVVFLFGLLFFTSGLIINLFQAFCFVLISPLSKNAYRRINRVFAELLPLEFLWLFWHCAGAKL
 KLFTDPETFRLMGKEHALVIINHKEILDWMVGWVLGQHLGCLGSILSVAKKSTKFLPVFGWSLWFSGYL
 FLERSWAKDKITLKSIESLKDYPLPFWLIIIFVEGTRFTRTKLLAAQQYAASSGLPVPNRNLIPTKGF

VSSVSHMRSFVPAIYDVTVAFFPKTSPPPTMLKLFEGQSVELHVHIKRHAMKDLPESSDDAVAQWCRDKFV
EKDALLDKHNSEDFTSGQEVHHVGRPIKALLVVISWVVVIFGALKFLLWSSLLSSWKGAFAFVIGLGI
VAGIVTLLMHILILSSQAEGSNPVKAAPAKLKTTELSSSKKVTNKEN

5 SEQ ID NO: 29 ChookLPAAT1

MAIPSAAVVFLFGLLFFTSGLIINLQAFCFVLISPLSKNAYRRINRVFAELLPLEFLWLFWHCAGAKL
KLFTDPETFRMLMGKEHALVIINHKIELDWMVGWVLGQHLGCLGSILSVAKKSTKFLPVFGWSLWFSEYL
FLERSWAKDKITLKSHIESLKDYPLPFWLIIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLIPTKGF
VSSVSHMRSFVPAIYDVTVAFFPKTSPPPTMLKLFEGQSVELHVHIKRHAMKDLPESSDDAVAQWCRDKFV
10 EKDALLDKHNSDFTSGQEVHHVGRPIKALLVVISWVVVIFGALKFLLWSSLLSSWKGAFAFVIGLGI
VAGIVTLLMHILILSSQAEGSNPVKAAPAKLKTTELSSSKKVTNKEN

SEQ ID NO: 30 ChookLPAAT2a

LSLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRINRVVAELLWLELVWLIDWWAGVKIKVFTDHETFNL
15 MGKEHALVVCNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDES
TLKSGKLRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLIPTKGFVSSVSHMRSFV
PAIYDVTVAIPKTSVPPTMLRIFKGQSSVLHVHLKRHLMKDLPESSDDAVAQWCRDIFVEKDALLDKHNA
EDTFSGQELQDIGRPIKSLLVVISWAVLVI FGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILF
SQSERSTPAKVAPAKPKNEGESSKTEMEKEH

20

SEQ ID NO: 31 ChookLPAAT2b

QIKVFTDHETFNLMGKEHALVVCNHKSDIDWLVGWVLAQWSGCLGSTLAVMKKSSKFLPVIGWSMWFSE
YLFLERSWAKDESTLKSGKLRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLIPTK
GFVSSVSHMRSFVPAIYDVTVAIPKTSVPPTMLRIFKGQSSVLHVHLKRHLMKDLPESSDDAVAQWCRDI
25 FVEKDALLDKHNAEDTFSGQELQDIGRPIKSLLVVISWAVLVI FGAVKFLQWSSLLSSWKGLAFSGIGL
GIVTLLMHILILFSQSERSTPAKVAPAKLKKEGESSKPEPDKQN

SEQ ID NO: 32 ChookLPAAT3a

LSLLFFVSGLIVNLVQAVCFVLIRPLLKNTYRRINRVVAELLWLELVWLIDWWAGIKIKVFTDHETFHL
30 MGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDES
TLKSGLNRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGFVSSVSQMRSFV
PAIYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHLMNDLPESDDAVAQWCRDIFVEKDALLDKHNA
EDTFSGQELQDTGRPIKSLLVVISWATLVVFGAVKFLQWSSLLSSWKGLAFSGIGLGIIITLLMHILILF
SQSERSTPAKVAPAKPKNEGESSKTEMEKEH

35

SEQ ID NO: 33 ChookLPAAT3b

LSLLFFVSGLIVNLVQAVCFVLIRPLLKNTYRRINRVVAELLWLELVWLIDWWAGIKIKVFTDHETFHL
MGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDES
TLKSGLNRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGFVSSVSQMRSFV
40 PAIYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHLMNDLPESDDAVAQWCRDIFVEKDALLDKHNA
EDTFSGQELQDIGRPIKSLLVVISWAVLEIFGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILF
SQSERSTPAKVAPAKPKKEGESSKPEPDKEN

SEQ ID NO: 34 CignealPAAT1

MAIAAAAVIFLFGLLFFASGIIINLQALCFVLIWPLSKNVYRRINRVFAELLMLDLCLFHWAGAKI
KLFTDPETFRMLMGMEHALVIMNHKTDLDWMVGWILGQHLGCLGSILSIKKSTKFIPLVGWSVWFSEYL
FLERSWAKDKSTLKSHMEKLKDYPLPFWLVI FVEGTRFTRTKLLAAQQYAASSGLPVPRNVLIPTKGF
VSCVSNMRSFVPAVYDVTVAFFPKSSPPPTMLKLFEGQSIVLHVHIKRHALKDLPESSDDAVAQWCRDKFV
EKDALLDKHNAEDTFSGQEVHHIGRPIKSLLVVIAWVVVIFGALKFLQWSSLLSTWKGAFAFVIGLGI
50 ATLLMHMLILSSQAERSNPAKPAK

SEQ ID NO: 35 CignealPAAT2

MAIAAAAVIFLFGLLFFASGIIINLFQALCFVLIWPLSKNVYRRINRVFAELLMLDCLFHWAGAKI
 5 KLFTDPETFRMLGMEHALVIMNHKTDLDWMVGWILGQHLGCLGSILSIKKSTKFI PVLGWSVWFSEYL
 FLERSWAKDESTLKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPKNVLI PRTKGF
 VSSVSHMRSFVPAIYDVTVAIPKTSAPPTLLRMFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFV
 EKDALLDKHNAEDTFSGQELHDI GRPVKSLLVVISWAMLVVFGAVKFLQWSSLLSSWKGLAFSGIGLGI
 ITLLMHILILFSQSERSSTPAKVAPAKQKNNEGESSKTEMEKEH

10 SEQ ID NO: 36 DcLPAAT1

SGLVVNLIQAFFFVLVRPFSKNAYRKINRVVAELLWLELIWLIDWWAGVKIQLYTDPETFKLMGKEHAL
 VICNHKSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDENTLKSGFQ
 RLRDFPHAFWLALFVEGTRFTQAKLLAAQYASSMGLPAPRNVLI PRTKGFVTAVTHMRPFVPAVYDVT
 LAIPKTSPPPTMLRLFKGQSSVVIHLKRHLMSDLPKSDDSVAQWCKDAFVVDNLLDKHKENDSFGDG
 15 VLQDTGRPLNSLVVISWACLLIFGALKFFQWSSILSSWKGLAFSAVGLGIVTVLMQILIQFSQSERSN
 RMPMSKHAK

SEQ ID NO: 37 DcLPAAT2

MAIPTAAYVVPLGAIFFFFSGLLVNLIQAFFFITVWPLSKKTYIRINKVIVELLWLEFVWLADWWAGLKI
 20 EVYADAETFQLMGKEHALVICNHKSDIDWLVGWILAQRAGCLGSSFAVTKKSAARYLPVVGWSIWFSGAI
 FLERSWEKIDENTLKAGFQRLREFPCAFLWGLFVEGTRFTQAKLLAAQYASTMGLPFPRNVLI PRTKGF
 IAAVNHMREFVPAIYDLTFAFPKDSPPPTMLRLKQPSVVHVHIKRHLMKDLPKNEAVAQWCKDVFL
 VKDKLLDKHKDDGSFGDGELHEIGRPLKSLVVTTWACLLILGTLKFLWSSLLSSWKGLIFSATGLAV
 LTVLMQFLIQSTQSERSNPASLSK

25

SEQ ID NO: 38 CcrLPAAT1a

LGLLFFISGLAVNLIQAVCFVFLRPLSKNTYRKINRVLAELLWLQVLVWLDWWAGVKIKVFADRESFNL
 MGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSSLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDES
 30 TLKEGLRRLKDFPRPFWLALFVEGTRFTQAKLLAAQYATSQGLPVPRNVLI PRTKVHVHVKRHLMKEL
 PETDEAVAQWCKDLFVEKDKLLDKHVAEDTFSDQPLQDIGRPVKPLLVSSWACLVAYGALKFLQWSSL
 LSSWKGIASVAVALAIVTILMQIMILFSQSERSIPAKVA

SEQ ID NO: 39 CcrLPAAT1b

LGLLFFISGLAVNLIQAVCFVFLRPLSKNTYRKINRVLAELLWLQVLVWLDWWAGVKIKVFADRESFNL
 MGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSSLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDES
 35 TLKEGLRRLKDFPRPFWLALFVEGTRFTQAKLLAAQYATSQGLPVPRNVLI PRTKGFVSASVSHMRSFV
 PAVYDMTVAIPKSSPSPTMLRLFKGQSSVVHVHVKRHLMKELPETDEAVAQWCKDLFVEKDKLLDKHVA
 EDTFSDQPLQDIGRPVKPLLVSSWACLVAYGALKFLQWSSLLSSWKGIASVAVALAIVTILMQIMILF
 SQSERSIPTKVA

40 SEQ ID NO: 40 CcrLPAAT2a

MAIAAAAVVFLFGLLFFTSGLIINLAQAVCFVLIWPLSKNAYRRINRVFAELLLELLWLFHWAGAKL
 KLFADPETFRFLFGKEHALVICNHRTDLDWMVGWVLGQHFGLGSILSVAKKSTKFLPVLGWSMWFSEYL
 FLERSWAKDKSTLKSHTERLKDYPLPFWLGI FVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PHTKLH
 45 VHIKRYAMKDLPESSDDAVAQWCRDIYVEKDAFLDKHNAEDTFSGQEVHHIGRPIKSLLVVISWVVVIF
 GALKFLRWSSLLSSWKGAFSVIGLGIVTLLVNILILSSQAERSNPAPAKLKTELSPPSKKVTNKEN

SEQ ID NO: 41 CcrLPAAT2b

MAIAAAAVVFLFGLLFFTSGLIINLAQAVCFVLIWPLSKNAYRRINRVFAELLLELLWLFHWAGAKL
 KLFADPETFRFLFGKEHALVICNHRTDLDWMVGWVLGQHFGLGSILSVAKKSTKFLPVLGWSMWFSEYL
 50 FLERSWAKDKSTLKSHTERLKDYPLPFWLGI FVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PHTKGF

VSSMSHMRSFVPAVYDLTVAFPKTSPPPTLLKLFEGQSVVLHVHIKRYAMKDLPESSDDAVAQWCRDIYV
EKDAFLDKHNAEDTFSGQEVHHIGRPIKSLLVVISWVVVIFGALKFLRWSSLLSSWKGAFSVIGLGI
VTLLVNILILSSQAERSNPAKVAPAKLKTLSKPKVTNKEN

5 SEQ ID NO: 42 BrLPAAT1a

AAAVIVPLGILFFISGLVNVLLQAICYVLIRPLSKNTYRKINRVVAETLWLELVWIVDWWAGVKIQVFA
DNETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVGWSMWFSEYLFLE
NWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELVPRNVLIPTKGFVSAV
SNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHVHIKCHSMKDLPESSDAIAQWCRDQFVAKDA
10 LLDKHIAADTFPGQEQNIGRPIKSLAVVLSWSCLLILGAMKFLHWSNLFSSWKGIASFALGLGIITLC
MQILIRSSQSERSTPAKVVPAPKPDNHNDSGSSSQTE

SEQ ID NO: 43 BrLPAAT1b

AAAVIVPLGILFFISGLVNVLLQAVCYVLVRPMSKNTYRKINRVVAETLWLELVWIVDWWAGVKIQVFA
15 DDETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVGWSMWFSEYLFLE
NWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELVPRNVLIPTKGFVSAV
SNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHVHIKCHSMKDLPESSDAIAQWCRDQFVAKDA
LLDKHIAADTFPGQEQNIGRPIKSLAVVLSWSCLLILGAMKFLHWSNLFSSWKGIASFALGLGIITLC
MQILIRSSQSERSTPAKVVPAPKPDNHNDSGSSSQTE

20

SEQ ID NO: 44 BrLPAAT1c

MAIAAAVIVPLGLLFFISGLLMNLLQAICYVLVRPLSKNTYRKINRVVAETLWLELVWIVDWWAGVKIK
VFADNETFSRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGSALAVMKKSSKFLPVGWSMWFSEYLF
LERNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLKAAQEYAASSELVPRNVLIPTKGFV
25 SAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHVHIKCHSMKDLPESSDAIAQWCRDQFVA
KDALLDKHIAADTFPGQEQNIGRPIKSLAVVLSWSCLLILGAMKFLHWSNLFSSWKGIASFALGLGII
TLCMQILIRSSQSERSTPAKVVPAPKPDNHNDSGSSSQTE

SEQ ID NO: 45 BjLPAAT1a

30 INLVVAETLWLELVWIVDWWAGVKIQVFADDET FNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGS
ALAVMKKSSKFLPVGWSMWFSEYLFLEARNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLK
AAQEYAASSELVPRNVLIPTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHVH
IKCHSMKDLPESSDAIAQWCRDQFVAKDALLDKHIAADTFPGQEQNIGRPIKSLAVSLIKTFPWLHPH
QLTNIFVLQVQVSWACLLTLGAMKFLHWSNLFSSWKGIASAFGLGIITLCMQILIRSSQSERSTPAK
35 VAPAKPK

SEQ ID NO: 46 BjLPAAT1b

INLVVAETLWLELVWIVDWWAGVKIQVFADDET FNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGS
ALAVMKKSSKFLPVGWSMWFSEYLFLEARNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLK
40 AAQEYAASSELVPRNVLIPTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHVH
IKCHSMKDLPEPEDEIAQWCRDQFVAKDALLDKHIAADTFPGQEQNIGRPIKSLAVVSWACLLTLGA
MKFLHWSNLFSSWKGIASAFGLGIITLCMQILIRSSQSERSTPAKVAPAKPK

SEQ ID NO: 47 BjLPAAT1c

45 INLVVAETLWLELVWIVDWWAGVKIQVFADDET FNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGS
ALAVMKKSSKFLPVGWSMWFSEYLFLEARNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLK
AAQEYAASSELVPRNVLIPTKGFVSAVSNMRSFVPAIYDMTVAIPKTSPPPTMLRLFKGQPSVVHVH
IKCHSMKDLPESSDAIAQWCRDQFVAKDALLDKHIAADTFPGQEQNIGRPIKSLAVVLSWSCLLILGA
MKFLHWSNLFSSWKGIASFALGLGIITLCMQILIRSSQSERSTPAKVVPAPKPDNHNDSGSSSQTE

50

SEQ ID NO: 48 BjlPAAT1d

INLVVAETLWLELVWVIDWWAGVKIQVFADDETFNRMGKEHALVVCNHRSDIDWLVGWILAQRSGCLGS
ALAVMKKSSKFLPVI GWSMWFSEYLFLEARNWAKDESTLKSGLQRLNDFPRPFWLALFVEGTRFTEAKLK
AAQEYAAASSELVPRNVLI PRTKGFVSAVSNMRSFVPAIYDMTVAI PKTSPPTMLRLFKGQPSVVHVH
5 IKCHSMKDLPESDDAIAQWCRDQFVAKDALLDKHIAADTFPGQQEQNIGRPIKSLAVSLS

SEQ ID NO: 49 CcLPAAT1a

MAIGVAAIVVPLGLLFILSGLMVNLIQAICFILVRPLSKNMYRRVNRVVVELLWLELIWLIDWWGGVKV
DVIADSETFQSLGKEHALVVSNNHRSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVI GWSMWFSEYV
10 FLERSWAKDESTLKSGLRRLKDFPRPFWLALFVEGTRFTQAKLLAAREYAASTGLPI PRNVLI PRTKGF
VSAVSNMRSFVPAIYDVTVAI PKTQPSPTMLRI FNRQPSVVHVHI KRHSMNQLPQTDEGVGQWCKDIFV
AKDALLDRHLAE

SEQ ID NO: 50 CcLPAAT1b

MAIGVAAIVVPLGLLFILSGLMVNLIQAICFILVRPLSKNMYRRVNRVVVELLWLELIWLIDWWGGVKV
DVIADSETFQSLGKEHALVVSNNHRSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVI GWSMWFSEYV
15 FLERSWAKDESTLKSGLRRLKDFPRPFWLALFVEGTRFTQAKLLAAREYAASTGLPI PRNVLI PRTKGF
VSAVSNMRSFVPAIYDVTVAI PKTQPSPTMLRI FNRQPSVVHVHI KRHSMNQLPQTDEGVAQWCKDIFV
AKDALLDRHLAEGKFDEKEFKRI RRP IKSLLVISSWSFLLMFGVFKFLKWSALLSTWKGVAVSTTVLLL
20 VTVMYMFILFSQSERSSPRKVAPSGPENG

SEQ ID NO: 51 UcLPAAT1a

MAIGVAAIVVPLGLLFILSGLIINLIQAICFILVRPLSKNMYRKVNRVVVELLWLELIWLIDWWGGVKV
DVIADSETFQSLGKEHALVVSNNHRSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVI GWSMWFSEYV
25 FLERSWAKDESTLKSGLQRLKDFPRPFWLALFVEGTRFTQAKLLAAQEYAASTGLPI PRNVLI PRTKGF
VSAVSNMRSFVPAIYDVTVAI PKTQPSPTMLRI FNRQPSVVHVHI KRHSMNQLPQTDEGVAQWCKDIFV
AKDALLDRHLAEGKFDEKEFKLI RRP IKSLLVISSWSFLLMFGVFKFLKWSALLSTWKGVAVSTAVLLL
VTVMYMFILFSQSERSSPRKVAPI GPENG

30 SEQ ID NO: 52 UcLPAAT1b

MAIGVAAIVVPLGLLFILSGLIINLIQAICFILVRPLSKNMYRKVNRVVVELLWLELIWLIDWWGGVKV
DVIADSETFQSLGKEHALVVSNNHRSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVI GWSMWFSEYV
FLERSWAKDESTLKSGLQRLKDFPRPFWLALFVEGTRFTQAKLLAAQEYAASTGLPI PRNVLI PRTKGF
VSAVSNMRSFVPAIYDVTVAI PKTQPSPTMLRI FNRQPSVVHVHI KRHSMNQLPQTDEGVAQWCKDIFV
35 AKDALLDRHLAE

SEQ ID NO: 53 LdLPAAT1

SLLFFMSGVLVNFIIQAVFYVLRPISKNTYRRINTLVAELLWLELVWVIDWWAGVKVQLYTDTESFRM
GKEHALLICNHRSDIDWLI GWVLAQRSGCLSSSIAMKKSSKFLPVI GWSMWFSEYLFLEARNWAKDENT
40 LKSGLQRLNDFPKPFWLALFVEGTRFTKAKLLAAQEYAAASAGLPVPRNVLI PRTKGFVSAVSNMRSFVP
AIYDLTVAI PKTTEQPTMLRLFRGKSSVVHVHLKRHLMKDLPKTDDGVAQWCKDQFISKDALLDKHVAE
DTFSGLEVQDIGRPMKSLVVVSWMCLLCLGLVKFLQWSALLSSWKGMMITTFVLGIVTVLMHILIRSS
QSEHSTPAK

45 SEQ ID NO: 54 CaequLPAAT1a

QRSGCLGSTLAVMKKSSKFLPVI GWSMWFSEYLFLEARNWAKDESTLKSGLRRLKDYPLPFWLALFVEGT
RFTQAKLLAAQQAASSGLPVPVPRNVLI PRTKGFVSSVSHMRSFVPAIYDVTVAI PKMSTPPTMLRI FKG
QSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLVVISW
AVLVIFGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKEGESSKT
50 ETEKEN

SEQ ID NO: 55 CaequLPAAT1b

5 DWWAGVKIKVFTDHE TL SLMGKEHALVISNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGW
SMWFSEYLFLE RSWAKDESTLKSG LKRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNV
LIPRTKGFVSSVSHMRSFVPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVA
QWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLV

SEQ ID NO: 56 CaequLPAAT1c

10 DWWAGVKIKVFTDHE TL SLMGKEHALVISNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGW
SMWFSEYLFLE RSWAKDESTLKSG LKRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNV
LIPRTKGFVSSVSHMRSFVPAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVA
QWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLVVISWAVLVI FGAVKFLQWSSLLSSWKGLA
FSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKEGESSKTETOKEN

15 SEQ ID NO: 57 CaequLPAAT1d

QRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLE RSWAKDESTLKSG LKRLKDYPLPFWLALFVEGT
RFTQAKLLAAQQYAASSGLPVPRNVLI PRTKGFVSSVSHMRSFVPAIYDVTVAIPKMSTPPTMLRIFKG
QSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRPVKSLLV

20 SEQ ID NO: 58 CglutLPAAT1a

LSLLFFVSGLFVN LVQAVCFVLIRPF SKNTYRRINRVVAELLWLELVWLIDWWAGVKIKVFTDHE TL SLMGKEHALVISNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLE RSWAKDES
TLKSG LKRLKDYPLPFWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLI PRTKGFVSSVSHMRSFV
PAIYDVTVAIPKMSTPPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDALLDKHNA
25 EDTFSGQELQDIGRPVKSLLVVISWAVLVI FGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILF
SQSERSTPAKVAPAKPKKEGESSKTETOKEN

SEQ ID NO: 59 CglutLPAAT1b

30 QAVCFVLIRPF SKNTYRRINRVVAELLWLELVWLIDWWAGVKIKVFTDHE TL SLMGKEHALVISNHKSD
IDWL VGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLE RSWAKDESTLKSG LKRLKDYPLP
FWLALFVEGTRFTQAKLLAAQQYAASSGLPVPRNVLI PRTKGFVSSVSHMRSFVPAIYDVTVAIPKMST
PPTMLRIFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDIGRP
VKSLLVVISWAVLVI FGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAK
PKKEGESSKTETOKEN

35

SEQ ID NO: 60 CprLPAAT1

MAIAAAAVVFLFGLLFFTSGLIINLAQAVCFVLIWPLSKNAYRRINRVFAELLLLLLELLWLFWHRAGAKL
KLFADPETFRLFGKEHALVICNHRTDL DWMVGWVLGQHFGCLGSILSVAKKSTKFLPVLGWSMWFSEYL
FLERSWAKDKSTLKSHTERLKDYPLPFWLGI FVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PHTKGF
40 VSSMSHMRSFVPAVYDLTVAFPKTSPPPTLLKLFEQGSSVLHVHIKRYAMKDLPESDDAVAQWCRDIYV
EKDAFLDKHNAEDTFSGQEVHHIGRPIKSLLVVISWVVI IFGALKFLRWSSLLSSWKGA FSVIGLGI
VTLLVNILILSSQAERSNPAKVPAKLKTELSPSKKVTNKEN

SEQ ID NO: 61 ChsLPAAT1

45 MAIPSAAVVFLFGLLFFASGLIINLVQAVCFVLIWPLSKNTCRRINIVFQDMLLSELLWLFWHRAGAKL
KFFTDPETYRHMKEHALVITNHR TDL DWMIGWVLGEHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYL
FLERNWAKDKSTFKSHIERLEDFFPQPFWFGIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PHTKGF
VSSVSHMRSFVPAVYETMTTFPKTSPPPTLLKLFEQGQPLVLHIHMKRHAMKDIPESDDAVAQWCRDKFV

EKDALLDKHNAEDTFGGLEVHIGRSIKSLMVVICWVVVVIIFGALKFLQWSSLLSSWKGIASGIGLGI
 NLLVHVHLILSSQAERSAPTKVAPAKLKTLLSSKKITNKEN

SEQ ID NO: 62 ChsLPAAT2

5 MAIPSAAVVFLFGLLFFASGLIINLVQAVCFVLIWPLSKNTCRRINIVFQDMLLSELLWLFHWRAGAKL
 KFFTDPEYTYRHMGEHALVITNHRDLDWMIGWVLGQHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYL
 FLERNWAKDKSTFKSHIERLEDFFPQPFWFGIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGF
 VSSVSHMRSFVPAIYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFV
 10 EKDALLDKHNAEDTFSGQELQDIGRPIKSLVVVISWAALVVFAGVKFLQWSSLLSSWKGLAFSGIGLGI
 ITLLMHILILFSQSERSTPAKVAPAKPKREGESSKTEMNKEN

SEQ ID NO: 63 CcalcLPAAT1a

MAIPAAAVVFLFGLLFFPSGLIINLVQAVCFVLIWPFSSRNTCRRINIVFQEMLLSELLWLFHWRAGAKL
 KLFADPEYTYRHMGEHALVITNHRDLDWMIGWALGQHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYL
 15 FWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGFVSCVSHMRSFVPAVYETTTMTFPKTS
 PPTLLKLFEGQPIVLHVHMKRHAMKDIPESEAVAQWCRDKFVEKDSLLDKHNAGDTFSCQEIHIGRPI
 KSLMVVISWVVVVIIFGALKFLQWSSLLSSWKGIASGIGLGIIVTLLVHILILSSQAERSTPAKVAPAKL
 KTELSSSTKVTNKEN

20 SEQ ID NO: 64 CcalcLPAAT1b

MAIPAAAVVFLFGLLFFPSGLIINLVQAVCFVLIWPFSSRNTCRRINIVFQEMLLSELLWLFHWRAGAKL
 KLFADPEYTYRHMGEHALVITNHRDLDWMIGWALGQHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYL
 FLERNWAKDKSTFKSHIERLEDFFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGF
 VSCVSHMRSFVPAVYETTTMTFPKTSPPPTLLKLFEGQPIVLHVHMKRHAMKDIPESEAVAQWCRDKFV
 25 EKDSLLDKHNAGDTFSCQEIHIGRPIKSLMVVISWVVVVIIFGALKFLQWSSLLSSWKGIASGIGLGI
 TLLVHILILSSQAERSTPAKVAPAKLTELSSSTKVTNKEN

SEQ ID NO: 65 CcalcLPAAT2

LSLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRINRVVAELLWLELVWLIDWWAGVKIKVFTDHTFRL
 30 MGTEHALVISNHKSDIDWLVGWVLAQRSGLGSTLAVMKSSKFLPVIWWSMWFSEYLFLEERSWAKDES
 TLKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGFVSSVSHMRSFV
 PAIYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDALLDKHNA
 EDTFSGQELQDIGRPIKSLVVVISWAALVVFAGVKFLQWSSLLSSWKGLAFSGIALGIITLLMHILILF
 SQSERSTPAKVAPAKPKKEGESSKTETDNKEN

35

SEQ ID NO: 66 ChtLPAAT1a

MAIPAAAVIFLFSILFFASGLIINLVQAVCFVLIWPLSKNTCRRINLVFQEMLLSELLGLFHWWRAGAKL
 KLYTDPETYPLLGKEHALMINHRDLDWMIGWVLGQHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYL
 FLERNWAKDKSTFKSHIERLEDFFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGF
 40 VSTVSHMRSFVPAVYDTTLTFPKTSPPPTLLNLFAGQPIVLHIHMKRHAMKDIPESEDDAVAQWCRDKFV
 EKDALLDKHNAEDAFSDQEFPIRSIKSLMVVISWVMVVIIFGALKFLQWSSLLSSWKGAFSVIAVGIV
 TLLMHISILSSQAERSNPAKVALPKLTELPSSTKVTNKEN

SEQ ID NO: 67 ChtLPAAT1b

MAIPAAAVIFLFSILFFASGLIINLVQAVCFVLIWPLSKNTCRRINLVFQEMLLSELLGLFHWWRAGAKL
 KLYTDPETYPLLGKEHALMINHRDLDWMIGWVLGQHLGCLGSILSVVKKSTKFLPVLGWSMWFSEYL
 FLERNWAKDKSTFKSHIERLEDFFPQPFWMAIFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGF
 VSTVSHMRSFVPAVYDTTLTFPKTSPPPTLLNLFAGQPIVLHIHMKRHAMKDIPESEDDAVAQWCRDKFV
 EKDALLDKHNAEDAFSDQEFPIRSIKSLMVVISWVMVVIIFGALKFLQWSSLLSSWKGIASGIGLGI
 50 TLLMHILILSSQAERSTPAKVAQAKVKTLPSSSTKVTNKEN

SEQ ID NO: 68 CwLPAAT1

MAIPAAAVIFLFGILFFASGLIINLVQAVCFVLIWPLSKNTCRRINLVFQEMLLSELLWLFHWRAGAEL
 5 KLFTDPETYRLLGKEHALVMTNHRDLDWMIGWVTGQHLGCLGSILSIKKSTKFLPVLGWSMWFSEYL
 FLERNWAKDKSTFKSHIERLEDFPQPFWMAlFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PHTKGF
 VSSVCHMRSFVPAVYDITLTFPKNSPPPTLLNLFAGQPIVLHIHIKRHAMKDMPKSDDAVAQWCRDKFV
 KKDALLDKHNTEDTFSDQEFPIGRPIKSLMVVISWVVVIFGTLKFLQWSSLLSSWKGLAFSGIGLGIV
 TLLVHILILSSQAERSTPPKVAPAKLKTLSSTTKVINKGN

10 SEQ ID NO: 69 CwLPAAT2b

LGLLFFVSGLIVNLVQAVCFVLIIRPLSKNTYRRLNRVVAELLWLELVWLIDWWAGVKIKVFTDHETFHL
 MGKEHALVICNHKSDIDWLVGWVLAQRSGLGSLTAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDES
 TLKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PRTKGFVSSVSHMRSFV
 PAIYDVTVAIPKTSPPPTMLRMFKGQSSVDALLDKHNADDTFSGQELHDIGRPIKSLLVVISWAVLVVF
 15 GAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLVHILILSSQAERSTSAKVAQAKVKTELSSSKVKKNKGN

SEQ ID NO: 70 CwLPAAT2a

LGLLFFVSGLIVNLVQAVCFVLIIRPLSKNTYRRLNRVVAELLWLELVWLIDWWAGVKIKVFTDHETFHL
 MGKEHALVICNHKSDIDWLVGWVLAQRSGLGSLTAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDES
 20 TLKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PRTKGFVSSVSHMRSFV
 PAIYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDVLLDKHNA
 EDTFSGQELQDIGRPVKSLLVVISWTLVIFGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILF
 SQSERSTPAKVAPAKPKKEGESSKMETDKEN

25 SEQ ID NO: 71 CgLPAAT1a

LAGWMGSSSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDESTLKSGNLRLKDYPLPFWLA
 LFVEGTRFTRAKLLAAQQYAASLGLPVPRNVLI PRTKGFVSSVSHMRSFVPAIYDVTVAIPKTSPPPTM
 IRMFKGQSSVLHVHLKRHVMDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSL
 LVVISWAVLEVFGAVKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPAKPKNE
 30 GESSKAEMEKEK

SEQ ID NO: 72 CgLPAAT1b

LAGWMGSSSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDESTLKSGNLRLKDYPLPFWLA
 LFVEGTRFTRAKLLAAQQYAASLGLPVPRNVLI PRTKGFVSSVSHMRSFVPAIYDVTVAIPKTSPPPTM
 35 IRMFKGQSSVLHVHLKRHVMDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSL
 LVRCFLVLSLIYLNIGIMLKLRGPCQLQVVISWAVLEVFGAVKFLQWSSLLSSWKGLAFSGIGLGIITLLM
 HILILFSQSERSTPAKVAPAKPKNEGESSKAEMEKEK

SEQ ID NO: 73 CgLPAAT1c

LAGWMGSSSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDESTLKSGNLRLKDYPLPFWLA
 LFVEGTRFTRAKLLAAQQYAASLGLPVPRNVLI PRTKGFVSSVSHMRSFVPAIYDVTVAIPKTSPPPTM
 IRMFKGQSSVLHVHLKRHVMDLPESDDAVAQWCRDIFVEKDALLDKHNAEDTFSGQELQDTGRPIKSL
 LVVTWAVLVISGAVKFLQWSSLLSSWKGLAFSGIGLGIVTLLMHILILFSQSERSTPAKVAPAKPKKE
 40 GESSKTEKDKEN

45

SEQ ID NO: 74 CpallPAAT1

LGLLFFVSGLIVNLVQAVCFVLIIRPLSKNTYRRLNRVVAELLWLELVWLIDWWAGVKIKVFTDHETLSL
 MGKEHALVICNHKSDIDWLVGWVLAQRSGLGSLTAVMKKSSKFLPVIGWSMWFSEYLFLEERSWAKDEN
 TLKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYATSSGLPVPRNVLI PRTKGFVSSVSHMRSFV

PAIYDVTVAIPKTSPPPTMLRMFKGQSSVLHVHLKRHLMKDLPESDDAVAQWCRDIFVEKDALLDKHNA
EDTFSGQELQDTGRPIKSLLVVISWAVLVIFGAVKFLQWSSLLSSWKGLAFSGVGLGIITLLMHILILF
SQSERSTPAKVAPAKPKDGESSKTEIEKEN

5 SEQ ID NO: 75 CaLPAAT1

MAIAAAAVIVPVSLFFVSGLIVNLVQAVCFVLIRPLFKNTYRRINRVFAELLWLELVWLIDWWAGVKI
KVFTDHETFHLMGKEHALVICNHKSDIDWLVGWVLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYL
FLERNWAKDESTLKSGNLRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLIPTKGF
VSSVSHMRSFVPAIYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHQMNDLPESDDAVAQWCRDIFV
10 EKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLVVFGAVKFLQWSSLLSSWKGLAFSGIGLGV
ITLLMHILILFSQSERSTPAKVAPAKPKIEGESSKTEMEKEH

SEQ ID NO: 76 CaLPAAT3

MTIASAAVVFLFGILLFTSGLIINLFQAFCSVLVWPLSKNAYRRINRVFAEFLPLEFLWLFWHWWAGAKL
15 KLFTDPETFRMLMGKEHALVIINHKEILDWVGWVVGQHLGCLGSILSVAKKSTKFLPVFGWSLWFSEYL
FLERNWAKDKKTLKSHIERLKDYPLPFWLIIFVEGTRFTRTKLLAAQQYAASAGLPVPRNVLIPTKGF
VSSVSHMRSFVPAIYDVTVAFPKTSPPPTMLKLFEHGFVELHVHIKRHAMKDLPESEDAVAQWCRDKFV
EKDALLDKHNAEDTFSGQEVHVGRIKSLLVVISWVVVIFGALKFLQWSSLLSSWKGLAFSVIGLGT
VALLMQILILSSQAERSIPAKETPANLKTLSKKVTNKEN

20

SEQ ID NO: 77 SalLPAAT1

MAIGAAAVVPLGLLFMLSGLMVNLIQAICFILVRPLSKNMYRRVNRVVVELLWLELIWLIDWWGGVKV
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25 VSAVSNMRSFVPAIYDVTVAIPKTSPPPTMLRIFNRQPSVVHVRIKRHSNMQLPPTDEGVAQWCKDIFV
AKDALLDRHLAEGKFDEKEFKRIRRPKSLLVISSWSFLLFGVFKFLKWSALLSTWKGVAVSTAVLLL
VTVVMYFILFSQSERSSPRKVAPSGPENG

SEQ ID NO: 78 CleptLPAAT1

MAIPAAVVIFFGLLFFSSGLIINLFQALCFVLIWPLSKNAYRRINRVFAELLSELLCLFDWWAGAKL
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YIERSWDKDKSTLKSHIERLKDYPLPFWLVIFAEGTRFTRTKLLAAQQYAASSGLPVPRNVLIPTKGF
VSCVNHMRSFVPAVYDLTVAFPKTSPPPTLLNLFEGQSVVLHVHIKRHAMKDLPESEDAVAQWCRDKFV
EKDALLDKHNAEDTFSSQEVHHTGSRPIKSLLVVISWVVVITFGALKFLQWSSWKGAFSVIGLGIVTL
35 LMHMLILSSQAERSKPAKVTQAKLKTLSISKKVTNKEN

SEQ ID NO: 79 ClopLPAAT1

MAIAAAAVIFLFGLLFFASGLIINLFQALCFVLIWPLSKNAYRRINRVFAELLSELLCLFDWWAGAKL
KLFTDPETLRLMGKEHALIIINHMTELDWMVGWVMGQHFGLGSII SVAKKSTKFLPVLGWSMWFSEYL
40 YLERSWAKDKSTLKSHIERLKDYPLPFWLVIIFVEGTRFTRTKLLAAQEYAASSGLPVPRNVLIPTKGF
VSCVNHMRSFVPAVYDVTVAFPKTSPPPTLLNLFEGRSIVLVHVIKRHAMKDLPESEDAVAQWCRDKFV
EKDALLDKHNAEDTFSGQEVHHTGRRPIKSLLVMSWVVVITFGALKFLQWSSWKGAFSVIGLGIVTL
LMHVILILSSQAERSNPAKVQAEINTELSISKKVTNKG

SEQ ID NO: 80 CcrasLPAAT1a

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLSELLCLFDWWAGAKL
KLFTDPETFRMLMGKEHALVIINHMTELDWMVGWVMGQHFGLGSILSVAKKSTKFLPVLGWSMWFTEYL
YIERSWDKDKSTLKSHIERLKDYPLPFWLVIIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLIPTKGF
VSSVSHMRSFVPAIYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRHVMDLPESDDAVAQWCRDIFV
EKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLEVFAGVFLQWSSLLSSWKGLAFSGIGLGI
50 ITLLMHILILFSQSERSTPAKVAPAKAK

SEQ ID NO: 81 CcrasLPAAT1b

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLSELLCLFDWWAGAKL
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 5 YIERSWDKDKSTLKSHIERLKDYPLPFWLVI FAEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PRTKGF
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 VKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPAKAK SEQ ID NO:

10 SEQ ID NO: 82 CcrasLPAAT1c

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLSELLCLFDWWAGAKL
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 15 EKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLEVFGAVKFLQWSSLLSSWKGLAFSGIGLGI
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SEQ ID NO: 83 CcrasLPAAT1d

MAIPAAAVIFLFGLIFFASGLIINLFQALCFVLIWPLWKNAYRRINRVFAELLSELLCLFDWWAGAKL
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 20 YIERSWDKDKSTLKSHIERLKDYPLPFWLVI FAEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PRTKGF
 VSSVSHMRSFVPAIYDVTVAIPKTSPPPTLIRMFKGQSSVLHVHLKRHVMDLPESDDAVAQWCRDIFV
 EKDALLDKHNAEDTFSGQELQDTGRPIKSLLVRCFLVLSLIYLNGLIILKLCGLCQVVISWAVLEVFGA
 VKFLQWSSLLSSWKGLAFSGIGLGIITLLMHILILFSQSERSTPAKVAPAKAKMEGESSKTEMEM

25

SEQ ID NO: 84 CkoeLPAAT1

MAIAAAPVIFLFGLLFFASGLIINLFQALCFVLIWPLSKNAYRRINRVFAELLSELLCLFDWWAGAKL
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 FLERNWAKDKRTLKSHIERMKDYPLPLWLILFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PHTKGF
 30 VSSVSHMRSFVPAIYDVTVAIPKTSPPPTLIRMFKGQSSVLHVHLKRHLMDLPESDDAVAQWCRDIFV
 EKDALLDKHNAEDTFSGQELQETGRPIKSLLVVISWAVLEVYGAVKFLQWSSLLSSWKGLAFSGIGLGL
 ITLLMHILILFSQSERSTPAKVAPAKPKKEGESSKTEMEM

SEQ ID NO: 85 CkoeLPAAT2

MHVLLMVTFRFSSFFVFDNVQALCFVLIWPLSKSAYRKINRVFAELLSELLCLFDWWAGAKLKLFTD
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 WAKDKRTLKSHIERMKDYPLPLWLILFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PHTKGFVSSVS
 HMRSFVPAVYDVTVAFPKTSPPPTMLSLFEGQSVVLHVHIKRHAMKDLPSDDAVAQWCRDKFVEKDAL
 LDKHNAEDTFSGQEVHHVGRPIKSLLVVISWMVVIIFGALKFLQWSSLLSSWKGAFAIGLGIATLLM
 40 HVLVVSQADRSNPAKVPPAKLNTLSSSKVTNKEN

SEQ ID NO: 86 pSZ2624 PmKASII

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CTGCATGCAATTGTCACAAGCGCTTACTCCAATTGTATTCGTTTGTCTG
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5 SEQ ID NO: 87 pSZ3204 *GarmFATA*

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[illegible]

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 AGCTCCAAAAAACTTGCCGCTCAAACCGCGTACCTCTGCTTTCGCGCAATC
 5 TGCCCTGTTGAAATCGCCACCACATTCATATTGTGACGCTTGAGCAGTCTG
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 CCCC GCGATGGGAAGAATCTCTCCCCGGGATGTGGGCCACCACCAGCAC
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 AGCAGGGGTTGCTAGGGATCGCTCCGAGTCCGCAAACCCTTGTCGCGTGG
 15 CGGGGCTTGTTTCGAGCTTgaagagc

SEQ ID NO: 88 pSZ4198 (*Bn*LPAT2)
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 TAAACCAGCACTGCAATGCTGATGCACGGGAAGTAGTGGGATGGGAACA

[illegible]

SEQ ID NO: 91 Nucleotide sequence of the GhomLPAT2A coding sequence, used in the transforming DNA from pSZ4412.

5 ATGgccatccccgccgcatcgtgatcggtgcccgtggcctgctgttctcatctccggcctgatcgtgaacctgctgcaggccct
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 10 ccaccgtggccgtgatgaagaagtccccaaggtgctgcccgtgatcggtggtccatgtggttctccgagtacctgttcttgga
 gcgcaactgggccaaggacgagtcaccctgaagtccggcctgcagcgctgcgcgactccccgcccttctggctggccct
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 tggacaagcacatcgccgaggacaccttctccgaccaggagctgcaggacatcgccgccccatcaagtccctggtggtgttc
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 tcgccgcccagagaagcacaagaacggcggtgtcccaggagatgggcccgcgagaagcagcacTGA
 15

SEQ ID NO: 92 Nucleotide sequence of the *GhomLPAT2B* coding sequence, used in the transforming DNA from pSZ4413.

20 ATGgagatccccgccgtggccgtgatcggtgcccacggcatcctgttctcatctccggcctgatcgtgaacctgatgcaggcca
 tctgcttctcctgatccgccccctgtccaagaacaccacgcgcatcgtgaaccgccagctggccgagctgctgtggctggagctg
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 gctcctgggccaaggacgagtcaccctgaagtccggcctgcagcgctgaaggactccccgcccttctggtggtggccctgtt
 25 cgtggagggcaccgcttcaccagggccaagctgctggccgcccaggagtacgcatgtccgcccgcctgcccgtgccccgca
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 gccccgccaagcccaacaaggccggcaactcctccgagaccgtgcgcgacaagcaccagTGA

SEQ ID NO: 93 Nucleotide sequence of the *GhomLPAT2C* coding sequence, used in the transforming DNA from pSZ4414.

35 ATGgccatccccgccgcatcatcgtgcccctggcctgatcttcttcaactccggcctgatcatcaacctgatccaggccgt
 gtgtacgtgctgatccgccccctgtccaagtcacacttcgcccgcacatcaaccgcgagctggccgagctgctgtggctggagctg
 gtgtgggtggtgactggtggccggcgctgaagatccagctgttaccgacaaggagacctgcactccatgggcaaggagc
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 40 cgtggagggcaccgcttcaccagcccaagctgctggccgcccaggagtacgccgctccgcccgcctgcccctccccgcaa
 cgtgctgatccccgcaccaagggttcgtgtccgctgaacatcatgcgctccttcgtgcccgccatctacgacgtgacctggtg
 ccatcccaagtctccccagcccaccatgctgcgctgttcaagggccagtctccgtggtgcacgtgcacctgaagcgcca
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 acaagtacgtggccgaggacaccttctccgaccaggagctgcaggacctgggcccgccatcaagtccctggtggtggtgacc
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gccgcctccctggccgtggtgaccgtgctgatgcagatcctgatccgcttctcccagtcgagcgctccacctccgccaagatcgc
cgccgccaagcgcaagaacgtggcgagcacTGA

SEQ ID NO: 94 Nucleotide sequence of the *GindPAT2A* coding sequence, used in the transforming DNA from pSZ4415.

5 ATGgccatccccgtggtggtggtgatcggtgcccgtgggctgctgttcttcatctccggcctgatcgtaacctgctgcaggccct
gtgcttcgtgctgatccgccccctgtccaagtccgcctaccgcaccatcaaccgccagctggtggagctgctgtggtgagctg
gtgtgcatcggtgactggtggcccggtgaagatccagctgttcatcgacaaggagacctgaactccatgggcaaggagc
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10 cgcaactgggccaaggacgagtcacacctgaagtccggcctgcagcgctgacgacttccccgcccccttctgggtggccctgt
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20 SEQ ID NO: 95 Nucleotide sequence of the *GindPAT2B* coding sequence, used in the transforming DNA from pSZ4416.

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35 SEQ ID NO: 96 Nucleotide sequence of the *GindPAT2C* coding sequence, used in the transforming DNA from pSZ4417.

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SEQ ID NO: 105 *CpalLPAAT1*

30 SEQ ID NO: 106 *CuPSR23 LPAAT2*

SEO ID NO: 107 *CkoeLPAAT1*

186

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15 SEQ ID NO: 108 CkoeLPAAT2

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SEQ ID NO: 111 ChookGPAT9-1

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SEQ ID NO: 113 *CignGPAT9-2*

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SEQ ID NO: 114 *CpalGPAT9-1*

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SEQ ID NO: 115 *CpalGPAT9-2*

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SEQ ID NO: 116 *CavigDGAT1*

SEQ ID NO: 117 *ChookDGAT1-1*

190

SEQ ID NO: 118 *CavigLPCAT*

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20

SEQ ID NO: 119 *CpalLPCAT*

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40

SEQ ID NO: 120 *CpauLPCAT*

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SEQ ID NO: 128 *Nucleotide sequence of transforming DNA contained in pSZ6383
TcDGAT1 and GarmFATA1(G108A)*

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[illegible]

45

SEQ ID NO: 129 *Nucleotide sequence of transforming DNA contained in pSZ6384
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[illegible]

35

SEQ ID NO: 130 *Nucleotide sequence of transforming DNA contained in pSZ6377 GarmFATA1(G108A)*

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SEQ ID NO: 131 Nucleotide sequence of transforming DNA contained in pSZ6315
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SEQ ID NO: 132 Nucleotide sequence of BnOTE (D124A) in pSZ6316:

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SEQ ID NO: 133 Nucleotide sequence of BnOTE (D209A) in pSZ6317:

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SEQ ID NO: 134 Nucleotide sequence of BnOTE (D124A, D209A) in pSZ6318

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 cgacggcgaggacctggtggtgacccacggcgacgcctgcctgcccacatcatggt
 ggagaacggccgcttctccggcttcatcgactgcggccgcctgggctggccgaccg
 ctaccaggacatcgccctggccaccgcgacatcgccgaggagctgggcggcgagtg
 15 ggccgaccgcttctcctggtgctgtacggcatcgccgcccccgactcccagcgcatcgc
 cttctaccgcctgctggacgagttcttctgaCAATTGACGCCCCGCGCGGCACCTG
 ACCTGTTCTCTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCG
 TGCATGATCGTCTCTGGCGCCCCGCGCGCGGTTTGTGCCCCCTCGCGGGCGCCGCGG
 CCGCGGGGGCGCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGC
 20 AGGAAGGCGTTGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAAC
 TAACAACAAAGGGTCGTTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCC
 GTGTAGAGGCTTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTT
 ACGACGTGGTAAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGG
 AGACGATCGGAAGCATCACAGCACAGGATCCCGCGTCTCGAACAGAGCGCGCAGAGG
 25 AACGCTGAAGGTCTCGCCTCTGTGCGACCTCAGCGCGGCATACACCACAATAACCAC
 CTGACGAATGCGCTTGGTTCTTTCGTCCATTAGCGAAGCGTCCGGTTCACACACGTGC
 CACGTTGGCGAGGTGGCAGGTGACAATGATCGGTGGAGCTGATGGTCGAAACGTTCA
 CAGCCTAGGGATATCGTGAAAACCTCGCTCGACCGCCCCGCGTCCCGCAGGCAGCGATG
 ACGTGTGCGTGACCTGGGTGTTTCGTGCGAAAGGCCAGCAACCCCAAATCGCAGGCCA
 30 TCCGGAGATTGGGATCTGATCCGAGCTTGGACCAGATCCCCACGATGCGGCACGGG
 AACTGCATCGACTCGGCGCGGAACCCAGCTTTCGTAAATGCCAGATTGGTGTCCGAT
 ACCTTGATTTGCCATCAGCGAAACAAGACTTCAGCAGCGAGCGTATTTGGCGGGCGT
 GCTACCAGGGTTGCATACATTGCCCATTTCTGTCTGGACCGCTTTACCGGCGCAGAG
 GGTGAGTTGATGGGGTTGGCAGGCATCGAAACGCGCGTGCATGGTGTGTGTCTGT
 35 TTTCGGCTGCACAATTTCAATAGTCGGATGGGCGACGGTAGAATTGGGTGTTGCGCT
 CGCGTGCATGCCCTCGCCCCGTCGGGTGTATGACCGGGACTGGAATCCCCCTCGCG
 ACCCTCCTGCTAACGCTCCCGACTCTCCCGCCCCGCGCGCAGGATAGACTCTAGTTCA
 ACCAATCGACAACCTAGTatggccaccgcatccacttctcggcggttcaatgcccgt
 gcggcgacctgcgtcgctcggcgggctccgggccccggcgcccagcgaggccctcc
 40 ccgtgcgcggggcgcgccatccccccccgcacatcgtgggtgtcctcctcctcctcca
 aggtgaaccccctgaagaccgaggccgtggtgtcctccggcctggccgaccgcctgc
 gcctgggctccctgaccgaggacggcctgtcctacaaggagaagttcatcgtgcgt
 gctacgaggtgggcatcaacaagaccgccaccgtggagaccatcgccaacctgctgc
 aggaggtgggctgcaaccacgcccagtcctggtgggtactccaccggcggttctcca
 45 ccacccccaccatgcgcaagctgcgcctgatctgggtgaccgcccgcacatgcacatcg
 agatctacaagtaacccgcctggtccgacgtggtggagatcgagtcctggggccagg
 gcgaggggaagatcggcacccgcccgcactggatcctgcgcgactacgccaccggcc
 aggtgatcgggccgcccacctccaagtgggtgatgatgaaccaggacacccgcccgc
 tgcagaaggtggacgtggacgtgcgcgacgagtacctggtgcactgcccccgcgagc
 50 tgccgctggccttccccgaggagaacaactcctccctgaagaagatctccaagctgg

5 aggaaccctcccagttactccaagctgggcctggtgccccgcccgcgcgcgacctggaca
 tgaaccagcacgtgaacaacgtgacctacatcggtggtgctggagtccatgcccc
 aggagatcatcgacacccacgagctgcagaccatcacccctggactaccgcccgcgagt
 gccagcacgacgacgtggtggactccctgacctcccccgagccctccgaggacgccc
 10 aggcggtgttcaaccacaacggcaccaacgggtccgccaacgtgtccgccaacgacc
 acggctgccgcaacttctgcacctgctgcgccctgtccggcaacggcctggagatca
 accgcgcccgacccgagtggtgcaagaagcccacccgcctggtactacaaggaccacg
 acggcgactacaaggaccacgacatcgactacaaggacgacgacgacaagtgaATCG
 ATgcagcagcagctcggatagtagtgcacacactctggacgctggctgctgtagtgac
 15 tggtgccgcccacacttgccttgacctgtgaatatccctgccgcttttatcaaac
 agcctcagtgtgtttgatcttgtgtgtacgcgcttttgcgagttgctagctgctgtg
 gctatttgcgaataaccacccccagcatccccttccctcggtttcataatcgcttgcatc
 ccaaccgcaacttatctacgctgtcctgctatcccctcagcgctgctcctgctcctgc
 tcactgcccctgcacagccttgggttgggctccgctgtattctcctggtactgca
 20 acctgtaaaccagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaa
 atgggaAAGCTTGAGCTCcagcgccatgccacgccctttgatggcttcaagtacgatt
 acgggtgttggtgtgtgtttgttgcgtagtgtgcatgggtttagaataatacacttg
 atttcttgcacaggaatctcggcttgcgcaggttcaaccccatcttcggagtct
 caggtcagccgcgcaatgaccagccgctacttcaaggacttgcacgacaacgcccag
 25 gtgagctatgtttaggacttgattggaaattgtcgtcgacgcataatcgcgctccgc
 gacagcaccacaagcaaaatgtcaagtgcgttccgatttgcgtccgcaggtcgatgtt
 gtgatcgctcggcgccggtatccgcccgtctgtcctgcgcttacgagctgaccaagcac
 cctgacgtccgggtacgcgagctgagattcgatttagacataaattgaagattaaacc
 cgtagaaaaatttgatggtcgcgaaactgtgctcgattgcaagaaattgatcgctcct
 30 ccactccgcaggtcgccatcatcgagcagggcggttgcctccggcgggcggcgctggc
 tggggggacagctgttctcggccatgtgtgtacgtagaaggatgaatttcagctgggt
 tttcggtgcacagctgtttgtgcatgatttgtttcagactattgttgaaatgttttta
 gatttcttaggatgcatgatttgtctgcatgcgact

30 **SEQ ID NO: 136 Amino acid sequence of Gm FATA wild-type parental gene;
 D3997, pSZ5083.**

35 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNNDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQSKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

40 **SEQ ID NO: 137 Amino acid sequence of Gm FATA S111A, V193A mutant gene; D3998,
 pSZ5084.**

45 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFaTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGKIGTRRDWILRDYATGQVIGRATSKWVMNNDTRRLQKV
 DaDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDPQSQSKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

**SEQ ID NO: 138 Amino acid sequence of Gm FATA S111V, V193A mutant gene; D3999,
 pSZ5085.**

5 MATASTFSAFNARCGDLRRSAGSGPRRPAPRPLPVRGRAIPPRIIVVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFvTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DaDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEI IDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 139 Amino acid sequence of *Gm* FATA G96A mutant gene; D4000, pSZ5086.

10 MATASTFSAFNARCGDLRRSAGSGPRRPAPRPLPVRGRAIPPRIIVVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVaCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEI IDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 15 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 140 Amino acid sequence of *Gm* FATA G96T mutant gene; D4001, pSZ5087.

20 MATASTFSAFNARCGDLRRSAGSGPRRPAPRPLPVRGRAIPPRIIVVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVtCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEI IDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 25 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 141 Amino acid sequence of *Gm* FATA G96V mutant gene; D4002, pSZ5088.

30 MATASTFSAFNARCGDLRRSAGSGPRRPAPRPLPVRGRAIPPRIIVVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVvCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEI IDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

35 **SEQ ID NO: 142 Amino acid sequence of *Gm* FATA G108A mutant gene; D4003, pSZ5089.**

40 MATASTFSAFNARCGDLRRSAGSGPRRPAPRPLPVRGRAIPPRIIVVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTaGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEI IDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTRMDYKDHDGDYKDHDIDYKDDDDK

45 **SEQ ID NO: 143 Amino acid sequence of *Gm* FATA L91F mutant gene; D4004, pSZ5090.**

MATASTFSAFNARCGDLRRSAGSGPRRPAPRPLPVRGRAIPPRIIVVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANfLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE

SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

5 **SEQ ID NO: 144 Amino acid sequence of *GmFATA* L91K mutant gene; D4005, pSZ5091.**

MATASTFSAFNARCGDLRRSAGSGPRRPARPLEVVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**k**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 10 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

15 **SEQ ID NO: 145 Figure 10. Amino acid sequence of *GmFATA* L91S mutant gene; D4006, pSZ5092. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the L91S residue is lower-case bold**

MATASTFSAFNARCGDLRRSAGSGPRRPARPLEVVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIAN**s**LQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 20 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

25 **SEQ ID NO: 146 Amino acid sequence of *GmFATA* G108V mutant gene; D4007, pSZ5093. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the G108V residue is lower-case bold.**

MATASTFSAFNARCGDLRRSAGSGPRRPARPLEVVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYST**v**GFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI GTRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 30 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

35 **SEQ ID NO: 147 Amino acid sequence of *GmFATA* T156F mutant gene; D4008, pSZ5094. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the T156F residue is lower-case bold.**

MATASTFSAFNARCGDLRRSAGSGPRRPARPLEVVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI **G**FRRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 40 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

45 **SEQ ID NO: 148 Amino acid sequence of *GmFATA* T156A mutant gene; D4009, pSZ5095. The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the T156A residue is lower-case bold.**

MATASTFSAFNARCGDLRRSAGSGPRRPARPLEVVRGRAIPPRIIVSSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKPAWSDVVEIESWGQEGGKI **Ga**RRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE

SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

- 5 SEQ ID NO: 149 Amino acid sequence of *GmFATA T156K* mutant gene; D4010, pSZ5096.
 The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the
 T156K residue is lower-case bold.

10 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKYPAWSDDVEIESWGQEGEKIG**k**RRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

15

- SEQ ID NO: 150 Amino acid sequence of *GmFATA T156V* mutant gene; D4011, pSZ5097.
 The algal transit peptide is underlined, the FLAG epitope tag is uppercase bold and the
 T156V residue is lower-case bold.

20 MATASTFSAFNARCGDLRRSAGSGPRRPAPRLPVRGRAIPPRIIVVSSSSSKVNPLKTEAVVSSGLADR
 LRLGSLTEDGLSYKEKFIVRCYEVGINKTATVETIANLLQEVGCNHAQSVGYSTGGFSTTPTMRKLRLI
 WVTARMHIEIYKYPAWSDDVEIESWGQEGEKIG**v**RRDWILRDYATGQVIGRATSKWVMNQDTRRLQKV
 DVDVRDEYLVHCPRELRLAFPEENNSSLKKISKLEDP SQY SKLGLVPRRADLDMNQHVNNVTYIGWVLE
 SMPQEIIDTHELQTITLDYRRECQHDDVVDLSLTSPEPSEDAEAVFNHNGTNGSANVSANDHGCRNFLHL
 LRLSGNGLEINRGRTEWRKKPTR**MDYKDHDGDYKDHDIDYKDDDDK**

25

- SEQ ID NO: 151 Nucleotide sequence of the *GmFATA S111A, V193A* mutant gene
 (D3998, pSZ5084). The promoter, 3'UTR, selection marker and targeting arms are the
 same as pSZ5083.

30 atggccaccgcacccactttctcggcggttcaatgcccgcgtgcggcgacactgcgtcgc
 tcggcggggctccgggccccggcgccccagcgaggccctccccgtgcgcgggcgcgcc
 atccccccccgcacatcgtggtgtcctcctcctcctccaaggtgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgctgcgcctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
 35 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtcctgtgggctactccaccggcggttcgccaccacccccaccatgcgc
 aagctgcgcctgatctgggtgaccgccccgcacatcgagatctacaagtacccc
 gcctgggtccgacgtggtggagatcgagtcctggggccaggggcgagggaagatcggc
 acccgccgcgactggatcctgcgcgactacgccaccggccagggtgatcgcccgcgcc
 40 acctccaagtgggtgatgatgaaccaggacaccgcgcctgcagaaggtggacgcg
 gacgtgcgcgacgagtagctggtgactgccccgcgagctgcgcctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
 tccaagctgggcctgggtgccccgcgcgcgcgacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggtggtgctggagtcctatgccccaggagatcatcgacacc
 45 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcggccgcaccgag

tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 152 Nucleotide sequence of the *GmFATA S111V, V193A* mutant gene

- 5 (D3999, pSZ5085). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083.

atggccaccgcatccacttttctcggcggttcaatgcccgctgcggcgacctgcgtcgc
tcggcggggtctccgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgcc
atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
10 accgaggccgtggtgtcctccggcctggccgaccgacctgcgcctgggctccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
cacgcccagtcctggtgggtactccaccggcggttctgtcaccacccccaccatgcgc
aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
15 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
accgcccgcgactggatcctgcgcgactacgccaccggccaggatgatcgcccgcgcc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgcy
gacgtgcgcgacgagtacctggtgcactgcccccgagctgcgcctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
20 tccaagctgggcctggtgccccgcccgcgcccacctggacatgaaccagcacgtgaac
aacgtgacctacatcggctgggtgctggagtcctatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
25 ctgcacctgctgcgcctgtccggcaacggcctggagatcaaccgcccgcacccgag
tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 153 Nucleotide sequence of the *GmFATA G96A* mutant gene (D4000,

- 30 pSZ5086). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083

atggccaccgcatccacttttctcggcggttcaatgcccgctgcggcgacctgcgtcgc
tcggcggggtctccgggccccggcgcccagcgaggccctccccgtgcgcgggcgcgcc
35 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
accgaggccgtggtgtcctccggcctggccgaccgacctgcgcctgggctccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtggcgtgcaac
cacgcccagtcctggtgggtactccaccggcggttctccaccacccccaccatgcgc
40 aagctgcgcctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
accgcccgcgactggatcctgcgcgactacgccaccggccaggatgatcgcccgcgcc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
gacgtgcgcgacgagtacctggtgcactgcccccgagctgcgcctggccttcccc
45 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
tccaagctgggcctggtgccccgcccgcgcccacctggacatgaaccagcacgtgaac
aacgtgacctacatcggctgggtgctggagtcctatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac

aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgccgcaacttc
ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

5

SEQ ID NO: 154 Nucleotide sequence of the *GmFATA* G96T mutant gene (D4001, pSZ5087). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083

10 atggccaccgcatccactttctcggcggttcaatgcccgtgctgcggcgacctgcgtcgc
tcggcggggtccggggccccggcgcccgagcgaggccctccccgtgctgcggggcgcgcc
atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccctgaag
accgaggccgtggtgtcctccggcctggccgaccgctgctgcctgggtccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgagggtgggcatc
15 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgacgtgcaac
cacgcccagtcctggtgggtactccaccggcggttctccaccacccccaccatgcgc
aagctgctgctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
accgcccgcgactggatcctgctgcgactacgccaccggccaggatgacggccgcgc
20 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
gacgtgctgcgacgagtagctggtgactgcccccgagctgctgctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
tccaagctgggcctggtgccccgcgcgcgcgacctggacatgaaccagcacgtgaac
aacgtgacctacatcggttgggtgctggagtcctatgccccaggagatcatcgacacc
25 cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctccccgagccctccgaggacgccgaggccgtgttcaaccac
aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgccgcaacttc
ctgcacctgctgctgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
30 cacgacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 155 Nucleotide sequence of the *GmFATA* G96V mutant gene (D4002, pSZ5088). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083

35 atggccaccgcatccactttctcggcggttcaatgcccgtgctgcggcgacctgcgtcgc
tcggcggggtccggggccccggcgcccgagcgaggccctccccgtgctgcggggcgcgcc
atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccctgaag
accgaggccgtggtgtcctccggcctggccgaccgctgctgcctgggtccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgagggtgggcatc
40 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtggtgtgcaac
cacgcccagtcctggtgggtactccaccggcggttctccaccacccccaccatgcgc
aagctgctgctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
accgcccgcgactggatcctgctgcgactacgccaccggccaggatgacggccgcgc
45 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
gacgtgctgcgacgagtagctggtgactgcccccgagctgctgctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
tccaagctgggcctggtgccccgcgcgcgcgacctggacatgaaccagcacgtgaac
aacgtgacctacatcggttgggtgctggagtcctatgccccaggagatcatcgacacc
50 cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg

gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgcccgaacttc
 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 5 cagcacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 156 Nucleotide sequence of the *GmFATA* G108A mutant gene (D4003, pSZ5089). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ50836.

10 atggccaccgcatccactttctcggcggttcaatgcccgtgctgcccgcacctgctgcgc
 tcggcggggtccgggccccggcgcccagcgaggccctccccgtgctgcccgcgcgc
 atccccccccgcgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgcctgctgcctgggtccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgagggtgggcatc
 15 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtcctggtggtactccaccgcccgttctccaccacccccaccatgcgc
 aagctgctgcctgatctgggtgaccgcccgcgcatgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
 acccgccgcgactggatcctgctgcgactacgccaccggccaggtgatcgccgcgcgc
 20 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
 gacgtgctgcgacgagtagctggtgactgccccgcgagctgctgcctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
 tccaagctgggcctggtgccccgcccgcgcccagctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtcctatgccccaggagatcatcgacacc
 25 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgcccgaacttc
 ctgcacctgctgctgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccaccgcgcatggactacaaggaccacgacggcgactacaaggac
 30 cagcacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 157 Nucleotide sequence of the *GmFATA* L91F mutant gene (D4004, pSZ5090). The promoter, 3'UTR, selection marker and targeting arms are the same as pSZ5083

35 atggccaccgcatccactttctcggcggttcaatgcccgtgctgcccgcacctgctgcgc
 tcggcggggtccgggccccggcgcccagcgaggccctccccgtgctgcccgcgcgc
 atccccccccgcgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgcctgctgcctgggtccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgagggtgggcatc
 40 aacaagaccgccaccgtggagaccatcgccaacttctgagaggtgggctgcaac
 cagccccagtcctggtggtactccaccgcccgttctccaccacccccaccatgcgc
 aagctgctgcctgatctgggtgaccgcccgcgcatgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
 acccgccgcgactggatcctgctgcgactacgccaccggccaggtgatcgccgcgcgc
 45 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
 gacgtgctgcgacgagtagctggtgactgccccgcgagctgctgcctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
 tccaagctgggcctggtgccccgcccgcgcccagctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtcctatgccccaggagatcatcgacacc
 50 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg

gtggactccctgacctcccccgagccctccgaggacgccgagggccgtgttcaaccac
 aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgcccgaacttc
 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccaccgcgatggactacaaggaccacgacggcgactacaaggac
 5 cagcacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 158 Nucleotide sequence of the *GmFATA* L91K mutant gene (D4005, pSZ5091).

atggccaccgcatccactttctcggcggttcaatgcccgtgctgcccgcacctgctgcgc
 10 tcggcggggtccgggccccggcgcccagcgaggccctccccgtgctgcccggcgcgcc
 atccccccccgcacatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgagggccgtggtgtcctcggcctggccgaccgcctgctgctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacaagctgcaggaggtgggctgcaac
 15 cagccccagtcctggtgggtactccaccggcggttctccaccacccccaccatgctgc
 aagctgctgctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
 acccgccgcgactggatcctgctgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
 20 gacgtgctgacgagtagtacctggtgactgccccgcgagctgctgctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
 tccaagctgggctggtgccccgcccgcgcccacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 25 gtggactccctgacctcccccgagccctccgaggacgccgagggccgtgttcaaccac
 aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgcccgaacttc
 ctgcacctgctgctgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccaccgcgatggactacaaggaccacgacggcgactacaaggac
 cagcacatcgactacaaggacgacgacgacaagtga
 30

SEQ ID NO: 159 Nucleotide sequence of the *GmFATA* L91S mutant gene (D4006, pSZ5092).

atggccaccgcatccactttctcggcggttcaatgcccgtgctgcccgcacctgctgcgc
 35 tcggcggggtccgggccccggcgcccagcgaggccctccccgtgctgcccggcgcgcc
 atccccccccgcacatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgagggccgtggtgtcctcggcctggccgaccgcctgctgctgggctccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaactcgtgctgcaggaggtgggctgcaac
 40 cagccccagtcctggtgggtactccaccggcggttctccaccacccccaccatgctgc
 aagctgctgctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
 acccgccgcgactggatcctgctgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
 45 gacgtgctgacgagtagtacctggtgactgccccgcgagctgctgctggccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
 tccaagctgggctggtgccccgcccgcgcccacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 50 gtggactccctgacctcccccgagccctccgaggacgccgagggccgtgttcaaccac

aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgccgcaacttc
 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
 cagcacatcgactacaaggacgacgacgacaagtga

5

SEQ ID NO: 160 Nucleotide sequence of the *GmFATA* G108V mutant gene (D4007, pSZ5093).

atggccaccgcatccactttctcggcggttcaatgcccgtgctgcgccgacctgcgtcgc
 10 tcggcggggtccggggccccggcgcccagcgaggccctccccgtgctgcgggcgcgcc
 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgctgctgctggtggtccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctgctgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 15 cagccccagtccgtgggctactccaccgtcggcttctccaccacccccaccatgcgc
 aagctgctgctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
 acccgccgcgactggatcctgctgctgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
 20 gacgtgctgctgacgagtacctggtgactgcccccgagctgctgctggtccttcccc
 gaggagaacaactcctccctgaagaagatctccaagctggaggaccctcccagtac
 tccaagctgggctggtgccccgcgcgcgacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggtggtgctggagtcctatgccccaggagatcatcgacacc
 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 25 gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
 aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgccgcaacttc
 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
 tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
 cagcacatcgactacaaggacgacgacgacaagtga

30

SEQ ID NO: 161 Nucleotide sequence of the *GmFATA* T156F mutant gene (D4008, pSZ5094).

atggccaccgcatccactttctcggcggttcaatgcccgtgctgcgccgacctgcgtcgc
 tcggcggggtccggggccccggcgcccagcgaggccctccccgtgctgcgggcgcgcc
 35 atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
 accgaggccgtggtgtcctccggcctggccgaccgctgctgctggtggtccctgacc
 gaggacggcctgtcctacaaggagaagttcatcgtgctgctgctgaggtgggcatc
 aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
 cagccccagtccgtgggctactccaccggcggttctccaccacccccaccatgcgc
 40 aagctgctgctgatctgggtgaccgcccgcacatcgagatctacaagtacccc
 gcctggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
 ttccgcccgcgactggatcctgctgctgactacgccaccggccaggtgatcgcccgcgcc
 acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaaggtggacgtg
 gacgtgctgctgacgagtacctggtgactgcccccgagctgctgctggtccttcccc
 45 gaggagaacaactcctccctgaagaagatctccaagctggaggaccctcccagtac
 tccaagctgggctggtgccccgcgcgcgacctggacatgaaccagcacgtgaac
 aacgtgacctacatcggtggtgctggagtcctatgccccaggagatcatcgacacc
 cagagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
 gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
 50 aacggcaccaacgggtccgccaacgtgtccgccaacgaccacgggtgccgcaacttc

ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

5 **SEQ ID NO: 162 Nucleotide sequence of the *GmFATA* T156A mutant gene (D4009, pSZ5095)**

atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcgccgacctgcgtcgc
tcggcggggtctccgggccccggcgccacgagaggccctccccgtgcgcgggcgcgcc
atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccctgaag
10 accgaggccgtggtgtcctccggcctggccgaccgctgcgctgggctccctgacc
gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
cacgcccagtcctggtgggtactccaccggcggttctccaccacccccaccatgcgc
aagctgcgctgatctgggtgaccgcccgcacatcgacatcgagatctacaagtacccc
15 gcctggtccgacgtggtggagatcgagtcctggggccaggggcgagggcaagatcggc
gcgcgcccgcgactggatcctgcgcgactacgccaccggccaggatcgcccgcgcc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
gacgtgcgcgacgagtacctggtgactgcccccgagagctgcgcctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
20 tccaagctgggcctggtgccccgcccgcgacacctggacatgaaccagcacgtgaac
aacgtgacctacatcggtggtgctggagtccatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctccccgagccctccgaggacgcccaggccgtgttcaaccac
aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
25 ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

30 **SEQ ID NO: 163 Nucleotide sequence of the *GmFATA* T156K mutant gene (D4010, pSZ5096).**

atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcgccgacctgcgtcgc
tcggcggggtctccgggccccggcgccacgagaggccctccccgtgcgcgggcgcgcc
atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccctgaag
accgaggccgtggtgtcctccggcctggccgaccgctgcgctgggctccctgacc
35 gaggacggcctgtcctacaaggagaagttcatcgtgcgctgctacgagggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
cacgcccagtcctggtgggtactccaccggcggttctccaccacccccaccatgcgc
aagctgcgctgatctgggtgaccgcccgcacatcgacatcgagatctacaagtacccc
gcctggtccgacgtggtggagatcgagtcctggggccaggggcgagggcaagatcggc
40 aagcgccgcgactggatcctgcgcgactacgccaccggccaggatcgcccgcgcc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
gacgtgcgcgacgagtacctggtgactgcccccgagagctgcgcctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtac
tccaagctgggcctggtgccccgcccgcgacacctggacatgaaccagcacgtgaac
45 aacgtgacctacatcggtggtgctggagtccatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctccccgagccctccgaggacgcccaggccgtgttcaaccac
aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgcccgaacttc
ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag

tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 164 Nucleotide sequence of the *GmFATA* T156V mutant gene (D4011, pSZ5097).

5 atggccaccgcatccacttttctcggcggttcaatgcccgtgctgcgggcgacctgcgtcgc
tcggcggggctccggggccccggcgcccgagcgaggccctccccgtgcgcgggcgcgcc
atccccccccgcatcatcgtggtgtcctcctcctcctccaaggatgaacccccctgaag
accgagggcgtggtgtcctcggcctggccgaccgctgcgctgggctccctgacc
10 gaggacggcctgtcctacaaggagaagttcatcgtgctgctacgaggtgggcatc
aacaagaccgccaccgtggagaccatcgccaacctgctgcaggaggtgggctgcaac
cacgcccagtcctggtgggctactccaccggcggttctccaccacccccaccatgcgc
aagctgcgctgatctgggtgaccgcccgcacatgcacatcgagatctacaagtacccc
gcttggtccgacgtggtggagatcgagtcctggggccaggcgagggaagatcggc
15 gtgcgccgcgactggatcctgcgcgactacgccaccggccaggatgacggccgcgc
acctccaagtgggtgatgatgaaccaggacacccgcccgcctgcagaagggtggacgtg
gacgtgcgcgacgagtagctggtgactgccccgcgagctgcgctggccttcccc
gaggagaacaactcctccctgaagaagatctccaagctggaggacccctcccagtag
tccaagctgggctggtgccccgcgcgcgacactggacatgaaccagcacgtgaac
20 aacgtgacctacatcggctgggtgctggagtccatgccccaggagatcatcgacacc
cacgagctgcagaccatcacctggactaccgcccgcgagtgccagcacgacgacgtg
gtggactccctgacctcccccgagccctccgaggacgccgaggccgtgttcaaccac
aacggcaccaacggctccgccaacgtgtccgccaacgaccacggctgccgcaacttc
ctgcacctgctgcgctgtccggcaacggcctggagatcaaccgcgccgcaccgag
25 tggcgcaagaagcccacccgcatggactacaaggaccacgacggcgactacaaggac
cacgacatcgactacaaggacgacgacgacaagtga

SEQ ID NO: 165 Amino acid sequence of wild type *BnOTE* in pSZ6315 (See SEQ ID NO: 131)

30 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRASQLRKPALDPLRAVISADQGSISPVNSCTPAD
RLRAGRLMEDGYSYKEKFIVRSYEVGINKTATVETIANLLQEVACNHVQKCGFSTDGFAATLTMRKLHLIW
VTARMHIEIYKPAWSDVVEIETWCQSEGRIGTRRDWILRDSATNEVIGRATSKWVMMNQDTRRLQRV
TDEVRDEYLVFCPREPRLAFPEENNSSLKKIPKLEDPAQYSMLELKPRRADLDMNQHVNNVTYIGWVLESI
PQEIIDTHELQVITLDYRRECQQDDIVDSLTTSEIPDDPISKFTGTNGSAMSSIQGHNESQFLHMLRLSENG
35 QEINRGRTQWRKKSSRMDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 166 Amino Acid sequence of *BnOTE* (D124A) in pSZ6316 (See SEQ ID NO: 132)

40 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRASQLRKPALDPLRAVISADQGSISPVNSCTPAD
RLRAGRLMEDGYSYKEKFIVRSYEVGINKTATVETIANLLQEVACNHVQKCGFSTAGFAATLTMRKLHLIW
VTARMHIEIYKPAWSDVVEIETWCQSEGRIGTRRDWILRDSATNEVIGRATSKWVMMNQDTRRLQRV
TDEVRDEYLVFCPREPRLAFPEENNSSLKKIPKLEDPAQYSMLELKPRRADLDMNQHVNNVTYIGWVLESI
PQEIIDTHELQVITLDYRRECQQDDIVDSLTTSEIPDDPISKFTGTNGSAMSSIQGHNESQFLHMLRLSENG
QEINRGRTQWRKKSSRMDYKDHDGDYKDHDIDYKDDDDK

45 SEQ ID NO: 167 Amino Acid sequence of *BnOTE* (D209A) in pSZ6317 (See SEQ ID NO: 133)

MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRASQLRKPALDPLRAVISADQGSISPVNSCTPAD
 RLRAGRLMEDGYSYKEKFIVRSYEVGINKTATVETIANLLQEACNHVQKCGFSTDGFAATLTMRKLHLIW
 VTARMHIEIYKPAWSDVVEIETWCQSEGRIGTRRDWILRDSATNEVIGRATSKWVMMNQDTRRLQRV
 TAEVRDEYLVFCPREPRLAFPEENNSSLKIPKLEDPAQYSMLELKPRRADLDMNQHVNNVTYIGWVLESI
 5 PQEIIDTHELQVITLDYRRECQDDIVDSLTTSEIPDDPISKFTGTNGSAMSSIQGHNESQFLHMLRLSENG
 QEINRGRTQWRKKSSRMDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 168 Amino acid sequence of BnOTE (D124A, D209A) in pSZ6318 (See SEQ ID NO: 134)

10 MATASTFSAFNARCGDLRRSAGSGPRRPARPLPVRGRASQLRKPALDPLRAVISADQGSISPVNSCTPAD
 RLRAGRLMEDGYSYKEKFIVRSYEVGINKTATVETIANLLQEACNHVQKCGFSTAGFAATLTMRKLHLIW
 VTARMHIEIYKPAWSDVVEIETWCQSEGRIGTRRDWILRDSATNEVIGRATSKWVMMNQDTRRLQRV
 TAEVRDEYLVFCPREPRLAFPEENNSSLKIPKLEDPAQYSMLELKPRRADLDMNQHVNNVTYIGWVLESI
 PQEIIDTHELQVITLDYRRECQDDIVDSLTTSEIPDDPISKFTGTNGSAMSSIQGHNESQFLHMLRLSENG
 15 QEINRGRTQWRKKSSRMDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 169 *CpauLPAAT*

MAIPAAAVIFLFGLLFFTSGLIINLFAQALCFVLVWPLSKNAYRRINRVFAE
 LLLSELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMGLW
 20 VMGQHLGCLGSILSVAKKSTKFLPVLGWSMWFSEYLYIERSWAKDRITLKS
 HIERLTDYPLPFWMVIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PRT
 KGFVSCVSHMRSFVPAVYDVTVAFPKTSPPPTLLNLFEGQSIVLHVHIKRH
 AMKDLPESDDAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHRTGSRPIKS
 LLVVISWVVVITFGALKFLQWSSWKGKAFSVIGLGIIVTLLMHMLILSSQAE
 25 RSSNPAKVAQAKLKTELSISKATDKEN

SEQ ID NO: 170 *CprocLPAATii*

MAIPAAAVIFLFGLIFFASGLIINLFAQALCFVLIWPI SKNAYRRINRVFAE
 LLLSELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVIINHMTELDWMVGW
 30 VMGQHFGCLGSILSVAKKSTKFLPVLGWSMWFTEYLYIERSWNKDKSTLKS
 HIERLKDYPLPFWLVI FAEGTRFTQTKLLAAQQYAASSGLPVPRNVLI PRT
 KGFVSCVSHMRSFVPAVYDLTVAFPKTSPPPTLLNLFEGQSVVLHVHIKRH
 AMKDLPESDDEVAQWCRDKFVEKDALLDKHNAEDTFSGQELQHTGRRPIKS
 LLVVISWVVVIAFGALKFLQWSSWKGKAFSVIGLGIIVTLLMHMLILSSQAE
 35 RSKPAKVAQAKLKTELSISKTVTDKEN

SEQ ID NO: 171 *CpaiLPAATI*

MAIPSAVVFLFGLLFFTSGLIINLFAQALCFVLISPLSKNAYRRINRVFAE
 LLPLEFLWLFHWCAGAKLKLFTDPETFRLMGKEHALVIINHKEILDWMVGW
 40 VLGQHLGCLGSILSVAKKSTKFLPVFGWSLWFSGYLFLERSWAKDKITLKS
 HIESLKDYPLPFWLII FVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PHT
 KGFVSSVSHMRSFVPAIYDVTVAFPKTSPPPTMLKLFEGQSVELHVHIKRH

AMKDLPESSDDAVAQWCRDKFVEKDALLDKHNSEDTFSGQEVHHVGRPIKAL
LVVISWVVVIIIFGALKFLLWSSLLSSWKGKAFSVIGLGIVAGIVTLLMHIL
ILSSQAEGSNPVKAAPAKLKTTELSSSKKVTNKEN

5 **SEQ ID NO: 172** *ChookLPAAT1*

MAIPSAAVVFLFGLLFFTSGLIINLFAQFCFVLIISPLSKNAYRRINRVFAE
LLPLEFLWLFWHCAGAKLKLFTDPETFRMLGKEHALVIINHKIELDWMVGW
VLGQHLGCLGSILSVAKKSTKFLPVFGWSLWFSEYLFLEERSWAKDKITLKS
HIESLKDYPLPFWLIIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PHT
10 KGFVSSVSHMRSFVPAIYDVTVAFPKTSPPPTMLKLFEGQSVELHVHIKRH
AMKDLPESSDDAVAQWCRDKFVEKDALLDKHNSEDTFSGQEVHHVGRPIKAL
LVVISWVVVIIIFGALKFLLWSSLLSSWKGKAFSVIGLGIVAGIVTLLMHIL
ILSSQAEGSNPVKAAPAKLKTTELSSSKKVTNKEN

15 **SEQ ID NO: 173** *CignLPAAT1*

MAIAAAAVIFLFGLLFFASGIIINLFAQALCFVLIWPLSKNVYRRINRVFAE
LLLMDLLCLFHWAGAKIKLFTDPETFRMLGMEHALVIMNHKTDLDWMVGW
ILGQHLGCLGSILSIAKKSTKFI PVLGWSVWFSEYLFLEERSWAKDKSTLKS
HMEKLKDYPLPFWLVIIFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PHT
20 KGFVSCVSNMRSFVPAVYDVTVAFPKSSPPPTMLKLFEGQSIVLHVHIKRH
ALKDLPESSDDAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHHIGRPIKSL
LVVIAWVVVIIIFGALKFLQWSSLLSTWKGKAFSVIGLGIATLLMHMLILSS
QAERSNPAKVAK

25 **SEQ ID NO: 174** *CavigLPAAT1*

MTIASAAVVFLFGILLFTSGLIINLFAQFCSVLVWPLSKNAYRRINRVFAE
FLPLEFLWLFWHWAGAKLKLFTDPETFRMLGKEHALVIINHKIELDWMVGW
VLGQHLGCLGSILSVAKKSTKFLPVFGWSLWFSEYLFLEERNWAKDKKTLKS
HIERLKDYPLPFWLIIFVEGTRFTRTKLLAAQQYAASAGLPVPRNVLI PHT
30 KGFVSSVSHMRSFVPAIYDVTVAFPKTSPPPTMLKLFEGHFVELHVHIKRH
AMKDLPESEDAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHHVGRPIKSL
LVVISWVVVIIIFGALKFLQWSSLLSSWKGIAFSVIGLGTVALLMQILILSS
QAERSIPAKETPANLKTTELSSSKKVTNKEN

35 **SEQ ID NO: 175** *CavigLPAAT2*

MAIAAAAVIVPVSLFFVSGLIVNLVQAVCFVLIRPLFKNTYRRINRVVAE
LLWLELVWLIIDWWAGVKIKVFTDHETFHLMGKEHALVICNHKSDIDWLVGW
VLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLFLEERNWAKDESTLKS
GLNRLKDYPLPFWLALFVEGTRFTRAKLLAAQQYAASSGLPVPRNVLI PRT
40 KGFVSSVSHMRSFVPAIYDVTVAIPKTSPPPTLLRMFKGQSSVLHVHLKRH
QMNDLPESDDAVAQWCRDI FVEKDALLDKHNAEDTFSGQELQDTGRPIKSL
LIVISWAVLVVFGAVKFLQWSSLLSSWKGIAFSVIGLGVITLLMHILILFS
QSERSTPAKVAPAKPKIEGESSKTEMEKEH

SEQ ID NO: 176 *CpalLPAAT1*

MAIAAAAVIVPLGLLFFVSGLIVNLVQAVCFVLIRPLSKNTYRRINRVVAE
 LLWLELVWLIDWWAGVKIKVFTDHETLSLMGKEHALVICNHKSDIDWLVGW
 5 VLAQRSGCLGSTLAVMKKSSKFLPVIGWSMWFSEYLPESDDAVAQWCRDIF
 VEKDALLDKHNAEDTFSGQELQDTGRPIKSLLVVISWAVLVI FGAVKFLQW
 SSSLSSWKGLAFSGVGLGIITLLMHILILFSQSERSTPAKVAPAKPKKDGE
 SSKTEIEKENVPGALLGQGREHPEVRPEPPEGLPPALLAGPVRGGHPLHPR
 QAAGRPAVRHLLRPARAPQRADPPHQGLRVLRVPHALLRARHLRRDRGHPQ
 10 DLPPP HHAAHVQGPVLRAARAPEAPPDEGP

SEQ ID NO: 177 *CuPSR23 LPAAT2*

MAIAAAAVIFLFGLIFFASGLIINLFQALCFVLIRPLSKNAYRRINRVFAE
 LLLSELLCLFDWWAGAKLKLFTDPETFRLMGKEHALVI INHMTELDWMVGW
 15 VMGQHFGCLGSIISVAKKSTKFLPVLGWSMWFSEYLYLERSWAKDKSTLKS
 HIERLIDYPLPFWLVI FVEGTRFTRTKLLAAQQYAVSSGLPVPRNVLI PRT
 KGFVSCVSHMRSFVPAVYDVTVAFPKTSPPPTLLNLFEGQSIMLHVHIKRH
 AMKDLPESDDAVAEWCRDKFVEKDALLDKHNAEDTFSGQEVCHSGSRQLKS
 LLVVISWVVVTTFGALKFLQWSSWKGAFAISAI GLGIVTLLMHVLI LSSQAE
 20 RSNPAEVAQAKLKTGLSISKVTDKEN

SEQ ID NO: 178 *CkoeLPAAT1*

MAIPAAVAVIPIGLLFIISGLIVNLIQAVVYVLIRPLSKNLHRKINKPIAE
 LLWLELIWLVDWWAGIKVEVYADSQTELMGKEHALLICNHRSDIDWLVGW
 25 VLAQRARCLGSALAIMKKS AKFLPVIGWSMWFSDYIFLDRTWAKDEKTLKS
 GFERLADFPMPFWLALFVEGTRFTKAKLLAAQEYAASRGLPV PQNVLI PRT
 KGFVTAVTHMRSYVPAIYDCTVDISK AHPAPSI LRLIRGQSSVVKVQITRH
 SMQELPETADGISQWCMDL FVTKDGFLEKYH SKDIFGSLPVQNI GRPVKSL
 IVVLCWYCLMAFGLFKFFMWSSLLSSWEGILSLGLILLAVAI VMQILIQST
 30 ESERSTPVKSIQKDP SKETLLQN

SEQ ID NO: 179 *CkoeLPAAT2*

MHVLLMVTFRFSSFFVFDNVQALCFVLIWPLSKSAYRKINRVFAELLSE
 LLCLFDWWAGAKLKLFTDPETFRLMGKEHALVI TNHKIDLDWMIGWILGQH
 35 FGCLGSVISIAKKSTKFLPIFGWSLWFSEYLF LERNWAKDKRTLKSHIERM
 KDYPLPLWLILFVEGTRFTRTKLLAAQQYAASSGLPVPRNVLI PHTKGFVS
 SVSHMRSFVPAVYDVTVAFPKTSPPPTMLSLFEGQSVVLHVHIKRHAMKDL
 PDSDDAVAQWCRDKFVEKDALLDKHNAEDTFSGQEVHHVGRPIKSLLVVIS
 WMVVIIIFGALKFLQWSSLLSSWKGAFAISAI GLGIATLLMHVLLVVF SQADRS
 40 NPAKVPPAKLNTELSSSKVTNKEN

SEQ ID NO: 180 *CprocLPAAT2*

- MAIPAAVAVIPIGLLFIIISGLIVNLIQAVVYVLIRPLSKNLYRKINKPIAE
 LLWLELIWLVDWWAGIKVEVYADSETLESMGKEHALLICNHRSDIDWLVGW
 VLAQRARCLGSALAIMKKSARKFLPVIGWSMWFSYIFLDRTWEKDEKTLKS
 GFERLADFPMPFWLALFVEGTRFTKAKLLAAQEFASRGLPVPQNVLI PRT
 5 KGFVTAVTHMRSYVPAIYDCTVDISKAPPSILRLIRGQSSVVKVQITRH
 SMQELPETPDGISQWCMDLFVTKDAFLEKYHSKDI FGSLPVHDIGRPVKSL
 IVVLCWYSLMAFGFYKFFMWSSLLSSWEGILSLGLVLIVIAIVMQILIQSS
 ESERSTPVKSVQKDPSKETLLQN
- 10 **SEQ ID NO: 181** *CavigGPAT9*
 MATGGSLKPSSSDLDLHPNIEDYLPSSGSSINEPAGKLRLRLDLDISPTLT
 EAAGAIVDDSFTRCFKSI PREPWNWNLYLFPLWCIGVLIRYFILFPGRVIV
 LTMGWITVISSFIAVRVLLKGHDALQIKLERLIVQLLCSSFVASWTGVVKY
 HGPRPSIRPKQVYVANHTSMIDFFILDQMTVFSVIMQKHGPGWVGLLQSTLL
 15 ESGVCIWFDRAEAKDRGIVAKKLWDHVGEGNNPLLI FPEGTCVNNNYSVM
 FKKGAFELGCTVCPVAIKYNKIFVDAFWNSKKQSFTRHLLQLMTSWAVVCD
 VWYLEPQTLKPGETPIEFAERVRDII SARAGLKKVPWDGYLKYSRPSPKHR
 ERKQQTFAESVLQRLEE
- 20 **SEQ ID NO: 182** *ChookGPAT9-1*
 MATAGSLKPSRSELDLDRPNIEDYLPSSGSSII EPAGKLRLRLDLDISPTLT
 EAAGAIVDDSFTRCFKSNPPEPWNWNIYLFPLWCFGVLI RYLILFPARVIV
 LTIGWIIIFLSSFIPVHLLLKGHDALRIKLERLLVELICSFVASWTGVVKY
 HGPRPSIRPKQVYVANHTSMIDFFILDQMTVFSVIMQKHGPGWVGLLQSTLL
 25 ESGVCIWFDRAEAKDRGIVAKKLWDHVGEGNNPLLI FPEGTCVNNNYSVM
 FKKGAFELGCTVCPVAIKYNKIFVDAFWNSKKQSFTRHLLQLMTSWAVVCD
 VWYLEPQTLKPGETPIEFAERVRDII SVRAGLKKVPWDGYLKYSRPSPKHT
 ERKQQNFAESVLQRLEKK
- 30 **SEQ ID NO: 183** *CignGPAT9-1*
 MATGGRLKPSSSELDLDRANTEDYLPSSGSSINEPVGKLRLRLDLDISPTLT
 EAAGAIVDDSFTRCFKSI PPEPWNWNIYLFPLWCFGVLI RYFILFPARVIV
 LTIGWITVISSFTAVRFLKGHNALQIKLERLIVQLLCSSFVASWTGVVKY
 HGPRPSIRPKQVYVANHTSMIDFLILDQMTVFSVIMQKHGPGWVGLLQSTLL
 35 ESGVCIWFNRAEAKDREIVAKKLWDHVGEGNNPLLI FPEGTCVNNHYSVM
 FKKGAFELGCTVCPVAIKYNKIFVDAFWNSRKQSFTMHLLQLMTSWAVVCD
 VWYLEPQTLKPGETAIEFAERVRDII SVRAGLKKVPWDGYLKYSRPSPKHR
 ESKQQSFAESVLRLEEK
- 40 **SEQ ID NO: 184** *CignGPAT9-2*
 MATGGRLKPSSSELDLDRANTEDYLPSSGSSINEPVGKLRLRLDLDISPTLT
 EAAGAIVDDSFTRCFKSI PPEPWNWNIYLFPLWCFGVLI RYFILFPARVIV
 LTIGWITVISSFTAVRFLKGHNALQIKLERLIVQLLCSSFVASWTGVVKY

HGPRPSIRPKQVYVANHTSMIDFLILDQMTVFSVIMQKHGPGWVGLLQSTLL
 ESVGCIWFNRAEAKDREIVAKKLWDHVGEGNNPLLI FPEGTCVNNHYSVM
 FKKGAFELGCTVCPVAIKYNKIFVDAFWNSKKHSFTRHLLQLMTSWAVVCD
 VWYLEPQTLKPGETPIEFAERVRDII SVRADLKKVPWDGYLKYSRPSPKHR
 5 ERKQQKFAESVLRRLLEEK

SEQ ID NO: 185 *CpalGPAT9-1*

MATAGRLKPSSSELELDLDRPNIEDYLPSSGSSINEPAGKLRLRDLLDISPM
 LTEAAGAIVDDSFTRCFKSIPPEPWNWNIYLFPLWCFGVLI RYLILFPARV
 10 IVLTVGWITVISSFITVRFLKGHDSLRIKLERLIVQLFCSSFVASWTGVV
 KYHGPRPSIRPQQVYVANHTSMIDFII LNQMTVFS AIMQKHGPGWVGLIQST
 ILESVGCIWFNRAEAKDREIVAKKLLDHVGEGNNPLLI FPEGTCVNNHYS
 VMFKKGAFELGCTVCPVAIKYNKIFVDAFWNSKKQSFTMHLLQLMTSWAVV
 CDVWYLEPQTLKPGETPIEFAERVRDII SVRAGLKKVPWDGYLKYSRPSPK
 15 HRERKQQSFAESVLRRLLEKR

SEQ ID NO: 186 *CpalGPAT9-2*

MATAGRLKPSSSELELDLDRPNIEDYLPSSGSSINEPAGKLRLRDLLDISPM
 LTEAAGAIVDDSFTRCFKSIPPEPWNWNIYLFPLWCFGVLI RYLILFPARV
 20 IVLTVGWITVISSFITVRFLKGHDSLRIKLERLIVQLFCSSFVASWTGVV
 KYHGPRPSIRPQQVYVANHTSMIDFII LNQMTVFS AIMQKHGPGWVGLIQST
 ILESVGCIWFNRAEAKDREIVAKKLLDHVGEGNNPLLI FPEGTCVNNHYS
 VMFKKGAFELGCTVCPVAIKYNKIFVDAFWNSKKLSFTMHLLQLMTSWAVV
 CDVWYLEPQTLKPGETPIEFAERVRDII SVRAGLKKVPWDGYLKYSRPSPK
 25 HRERKQQTFAESVLRRLLEEKGNVPTVN

SEQ ID NO: 187 *CavigDGAT1*

MAIADGGIIGAAGSISALTADTDPPSLRRRNVPAGQASAVSAFSTESMAKH
 LCDPSREPSPPKSSDDGKDPDIGSVDSLNEKPSSPAAGKGRLQHDLRFTY
 30 RASSPAHRKVKEsplSSSNI FKQSHAGLFNLCVVVLVAVNSRLIIENLMKY
 GLLIKTGFWFSSRSLRDWPLFMCCLSLPI FPLAAFLVEKLAQKNRLQEPTV
 VCCHVLITSVSI LYPVLVILRCDSAVLSGVALMLFACIVWLKLVSYAHSNY
 DMRYVAKSLDKGEPVVDSDVIADHPYRV DYKDLVYFMVAPTLCYQLSYPLTP
 CVRKSWIARQVMKLVLF TGVMGFIVEQYINPIVQNSKHPLKGDLLYAIERV
 35 LKLSVPNLYVWLCMFYCFHLWLNILAE LICFGDREFYKDWWNAKTVEEYW
 RMWNMPVHKWVMVRHIYFPCLRNGIPRGVAVLIAFLVSAVFHEL CIAVPCHV
 FKLWAFIGIMFQVPLVLVSNCLQKKFQSSMAGNMFFWFI FCIFGQPMCVLL
 YYHDL MNRKGSRID

SEQ ID NO: 188 *ChookDGAT1-1*

MAIADGGSAGAAGSISGSDPSPSTAPSLRRRNASAGQAFSTESMARDLCDP
 SREPSLSPKSSDDGKDPADDIGAADSVDSGGVKDEKPSSQAAAKARLEHDL
 RFTYRASSPAHRKVKEsplSSSNI FKQSHAGLFNLCVVVLVAVNSRLIIEN

LMKYGLLIKTFWFSSRSLRDWPLFMCCLSLPIFPLAAFLVEKLAQKNRLQ
 EPTVVCCHVIIITSVSILYPVLVILRCDSAVLSGVALMLFACIVWLKLVSYA
 HANYDMRSVAKSLDKGETVADSVIVDHPYRVDYKDLVYFMVAPTLCYQLSY
 PLTPYVRKSWVARQVMKLVLFTGVMGFIVEQYINPIVQNSKHPLKGDLLYA
 5 IERVLKLSVPNLYVWLCMFYCFHLLWLNILAEITCFGDREFYKDWWNAKTV
 EEWYRMWNMPVHKWMVRHIYFPCLRNGIPRGVAVLIAFLVSAVFHELICIAV
 PCHVFKLWAFIGIMFQVPLVLVSNCLQKKFQSSMAGNMFFWFI FCI FGQPM
 CVLLYYHDLNMRKGSRID

10 **SEQ ID NO: 189** *CavigLPCAT*

MGLVSVAAAIGVSVPPVARFLLCFLATIPVSFLWRLVPGRLPKHLYSAASGA
 ILSYLSFGASSNLHFIVPMTLGYSMLFFRPFSGLLTFFLGFGYLIGCHVY
 YMSGDAWKEGGIDATGALMVLTCLKVISCSMNNDGLLKEEGLRESQKKNRL
 TKMPSLIEYFGYCLCCGSHFAGPVYEMKDYLEWTEGKGIWSRSQKEPKPSP
 15 FGGALRAIIQAAVCMAMYLYLVPHHPLTRFTEPVYYEWGFFRRLSYQYMAA
 LTARWKYYFIWSISEASLIISGLGFSGWTESSPPKPRWDRAKNVDIIGVEF
 AKSSVQLPLVWNIQVSIWLRHYVYDRLVQNGKRPGFFQLLATQTVSAVWHG
 LYPGYIIFFVQSALMIAGSRVIYRWQQAVPPKMGLVKNI FVFFNFAYTLLV
 LNYSAVGFMVLSMHETLASYGSVYYIGTILPITLILLSYVIKPGKPARSKA
 20 HKEQ

SEQ ID NO: 190 *CpalLPCAT*

MELGSVAAAIGVSVPPVARFLLCFLATIPVSFLWRLVPGRLPKHLYSAASGA
 ILSYLSFGPSSNLHFIVPMTLGYSMLFFRPFSGLLTFFLGFGYLIGCHVY
 25 YMSGDAWKEGGIDATGALMVLTCLKVISCSINNDGLLKEEGLRESQKKNRL
 TKMPSLIEYIGYCLCCGSHFAGPVYEMKDYLEWTEGKGVWSHSEKEPKPSP
 FGGALRAIIQAAVCMAMMYLVPHHPLSRFTEPVYYEWGFFRRLSYQYMAA
 LTARWKYYFIWSISEASLIISGLGFSGWTESSPPKPRWDRAKNVDIIGVEF
 AKSSVQLPLVWNIQVSTWLRHYVYDRLVQNGKRPGFFQLLATQTVSAIWHG
 30 LYPGYIIFFVQSALMIAGSRVIYRWQQAVPPKMGLVKNI FVFFNFAYTLLV
 LNYSAVGFMVLSMHETLASYGSVYYIGTILPITLILLSYVIKPGKPARSKA
 HKEQ

SEQ ID NO: 191 *CpauLPCAT*

MELEIGSVAAAIGVSVPPVARFLLCFLATIPVSFLCRLPARLPKHLYSAAS
 GAILSYLSFGPSSNLHFIVPMSLGYSMLFFRPFSGLLTFFLGFGYLIGCH
 VYYMSGDAWKEGGIDATGALMVLTCLKVISCSINNDGLLKEEGLRESQKKN
 RLTKMPSLIEYFGYCLCCGSHFAGPVYEMKDYLEWTEGKGIWSRSEKDPKP
 SPFGGALRAIIQAAVCMAMHMYLVPHHPLTRFTEPVYYEWGFFRRLSYQYM
 40 AAQTARWKYYFIWSISEASLIISGLGFSGWTESSPPKPRWDKAKNVDIIGV
 EFAKSSVQLPLVWNIQVSTWLRHYVYDRLVQNGKRPGFFQLLATQTVSAVW
 HGLYPGYIIFFVQSALMIAGSRVIYRWQQAVPQKMGLVKNI FVFFNFAYTL

LVLNYSAVGFMVLSMHETLASVGSVYYIGTILPITLILLSYVIKPGKPTRS
KVHKEQ

SEQ ID NO: 192 *CschuLPCAT*

5 MELEMEPLAAAI GVSVA VFRFLVCFIATIPVSFICRLVPGGLPRHLFSAAS
GAVLSYLSFGFSSNLHFLVPM TLGYLSMILFRRFCGILTF FLGFGYLIGCH
VYYMSGDAWKEGGIDATGALMVLT LKVISCSINYNDG LLKEEGLRESQKKN
RLIRLPSLIEYFGYCLCCGSHFAGPVYEMKDYL DWTEGKGIWSHSEKGP KP
SPLRAALRAIIQAGFCMAMYLYLVPHYPLTRFTDPVYYEWGILRRLSYQYM
10 ASFTARWKYYFIWSISEASLIISGLGFGSGWTESSPPKPRWDRAKNVDILGV
ELAKSSVQIPLVWNIQVSTWLRHYVYDRLVQNGKRPGFLQLLATQTVSAIW
HGVYPGYLIFFVQSALMIAGSRAIYRWQQAVPPKMSLVKNTLVFFNFAYTL
LVLNYSAVGFMVLSMHETLASVGSVYYVGTILPVT LILLGYVIKPGKSPRS
KASKEQ

15

SEQ ID NO: 193 *CavigPLA2-1*

MNFDFLSNI PWFGAKASDNAGSSFGSATIVIQQPPPVSRGFDIRHWGWPWS
VLSVLPWGKPGCDEL RAPPTTINRRLKRNATSMHSSAVRGNAEAARVRFRP
YVSKVPWHTGFRGLLSQLFPRYGHYCGPNWSSGKN GGS PVWDQRPIDWLDY
20 CCYCHDIGYDTHDQAKLLEADLAFLECLERPSYPTKGDAHVAHMYKTMCVT
GLRNVLI PYRTQLLR LNSRQPLIDFGWLSNAAWKGWNAQKS

SEQ ID NO: 194 *CignPLA2-1*

MNLDFLSKI PWFEAKASENPGLNLGSTTIVIKQPRQGFDIRHWGWPWSVLT
25 WGNRVTDDEVHAPPTTINRRLKRNATGPAVQGDTEAARLRFRPYVSKVPWHT
GFRGLLSQLFPRYGHYCGPNWSSGKN GGS PVWDQRPIDWLDYCCYCHDIGY
DTHDQAKLLEADLAFLECLERPSYPTTGDAHVAHMYKTMCVTGLRNVLI PY
RTQLLRLNFRQPLIDFGWLSNAAWKGWSAQKT

30 **SEQ ID NO: 195** *CuPSR23PLA2-2*

MVHLPHTLKLGLVIAISISGLCFSSTPARALNVGIQAAGVTVSVGKGCSRK
CESDFCKVPPFLRYGKYCGLMYSGCPGEKPCDGLDACC MKHDACVQAKNND
YLSQECSQNLLNCMASFRMSGGKQFKGSTCQVDEVVDVLT VVMEAAALLAGR
YLHKP

35

SEQ ID NO: 196 *CprocPLA2-2*

MVHLPHTLKLGLVIAISISGLCLSSTPARALNVGIQAAGVTVSVGKGCSRK
CESDFCKVPPFLRYGKYCGLMYSGCPGEKPCDGLDACC MKHDACVQAKNND
YLSQECSQNLLNCMASFRMSGGKQFKGSTCQVDEVVDVLT VVMEAAALLAGR
40 YLHKP

SEQ ID NO: 197 *pSZ4329*

agcgggaagagcgcccaatgtttaaacccctcaactgcgacgctgggaaccttctccgggcaggcgatgtgctgggtttgcctcctt
ggcacggctctacacgctcgagtacgcatgaggcggtgatggctgtgtcggttgccacttctccagagacggcaagtctgcat
cctctgctgtgtggcgacgctgcagcagtcctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacaca
atccatttttctaaagcaaatgactgctgattgaccagatactgtaacgctgatttcgctccagatcgacagatagcgaccatgtt
5 gctgctgtgaaaatctggattccgaattcgaccctggcgctccatccatgcaacagatggcgacacttggtacaattcctgtcaccc
atggcatggagcaggtccacttagattccgatcaccacgcacatctcgtataatagtcattcgttctgtcttcgatcaatctcaa
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35 SEQ ID NO: 198 pSZ5078

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accgct

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WHAT IS CLAIMED:

1. A recombinant vector construct or a host cell comprising nucleic acids that encodes a protein having acyltransferase activity, wherein the amino acid
5 sequence of the acyltransferase has at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172,
10 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.
2. The recombinant of claim 1, wherein the amino acid sequence of the protein comprises:
 - 15 a. at least 96.3% identity to an acyltransferase of clade 1 of Table 5;
 - b. at least 93.9% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 86.5% identity to an acyltransferase of clade 3 of Table 5; or
 - d. at least 78.5% identity to an acyltransferase of clade 4 of Table 5.
- 20 3. The recombinant vector construct or a host cell of claim 1, wherein the amino acid sequence of the protein comprises:
 - a. at least 98% identity to an acyltransferase of clade 1 of Table 5;
 - b. at least 98% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 90% identity to an acyltransferase of clade 3 of Table 5; or
 - 25 d. at least 80% identity to an acyltransferase of clade 4 of Table 5.
4. The recombinant vector construct or a host cell of claim 1, wherein the amino acid sequence of the protein comprises:
 - a. at least 99% identity to an acyltransferase of clade 1 of Table 5;
 - 30 b. at least 99% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 95% identity to an acyltransferase of clade 3 of Table 5; or
 - d. at least 85% identity to an acyltransferase of clade 4 of Table 5.
5. The recombinant vector construct or a host cell of claim 1, wherein the amino acid sequence of the protein comprises:
 - 35 a. at least 98% identity to an acyltransferase of clade 3 of Table 5; or
 - b. at least 90% identity to an acyltransferase of clade 4 of Table 5.
6. The recombinant vector construct or a host cell of claim 1, wherein the amino acid sequence of the protein comprises:
 - 40 a. at least 99% identity to an acyltransferase of clade 3 of Table 5; or

- b. at least 95% identity to an acyltransferase of clade 4 of Table 5.
7. The recombinant vector construct or a host cell of claim 1, wherein the amino acid sequence of the protein comprises at least 98% or 99% identity to an acyltransferase of clade 4 of Table 5.
8. The nucleic acids of any of claims 1 to 7, wherein the nucleic acids encoding the acyltransferase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.
9. The nucleic acid of claim 8, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
10. The nucleic acid of claim 8, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
11. The host cell of any of claims 1 to 10, wherein the cell is a microalgal cell, microbial cell or a plant cell, and wherein the fatty acid profile or the sn-2 profile of the host cell is altered by the expression of the nucleic acids.
12. The host cell of claim 11, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
13. The host cell of claim 12, wherein the cell is a *Prototheca moriformis* cell.
14. The recombinant vector construct or a host cell of any of claims 1 to 13, wherein the acyl transferase is a lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2).
15. The recombinant vector construct or a host cell of claim 14, wherein the acyl transferase is lysophosphatidic acid acyltransferase (LPAAT).
16. A method of cultivating a host cell, the host cell comprising recombinant nucleic acids encoding a protein having acyltransferase activity, wherein the amino acid sequence of the acyltransferase has at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48,

49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.

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17. The method of claim 16, wherein the amino acid sequence of the protein comprises:
 - a. at least 96.3% identity to an acyltransferase of clade 1 of Table 5;
 - b. at least 93.9% identity to an acyltransferase of clade 2 of Table 5;
 - 10 c. at least 86.5% identity to an acyltransferase of clade 3 of Table 5; or
 - d. at least 78.5% identity to an acyltransferase of clade 4 of Table 5.
18. The method of claim 16, wherein the amino acid sequence of the protein comprises:
 - 15 a. at least 98% identity to an acyltransferase of clade 1 of Table 5;
 - b. at least 98% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 90% identity to an acyltransferase of clade 3 of Table 5; or
 - d. at least 80% identity to an acyltransferase of clade 4 of Table 5.
- 20 19. The method of claim 16, wherein the amino acid sequence of the protein comprises:
 - a. at least 99% identity to an acyltransferase of clade 16 of Table 5;
 - b. at least 99% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 95% identity to an acyltransferase of clade 3 of Table 5; or
 - 25 d. at least 85% identity to an acyltransferase of clade 4 of Table 5.
20. The method of claim 16, wherein the amino acid sequence of the protein comprises:
 - a. at least 98% identity to an acyltransferase of clade 3 of Table 5; or
 - 30 b. at least 90% identity to an acyltransferase of clade 4 of Table 5.
21. The method of claim 16, wherein the amino acid sequence of the protein comprises at least 99% identity to an acyltransferase of clade 3 of Table 5; or
 - b. at least 95% identity to an acyltransferase of clade 4 of Table 5.
- 35 22. The method of claim 16, wherein the amino acid sequence of the protein comprises:
 - a. at least 98% or 99% identity to an acyltransferase of clade 4 of Table 5.
- 40 23. The method of any of claims 16 to 22, wherein the nucleic acids encoding the acyltransferase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding

sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.

24. The method of claim 23, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
25. The method of claim 23, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
26. The method of any of claims 16 to 25, wherein the cell is a microalgal cell, microbial cell or a plant cell.
27. The method of claim 26, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
28. The method of claim 27, wherein the cell is a *Prototheca moriformis* cell.
29. The method of any of claims 16 to 28, wherein the acyl transferase is a lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2).
30. The method of claim 29, wherein the acyltransferase is lysophosphatidic acid acyltransferase (LPAAT).
31. A method of producing a triglyceride oil in a host cell, the host cell comprising recombinant nucleic acids encoding a protein having acyltransferase activity, wherein the amino acid sequence of the acyltransferase has at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46, 47, 48, 49, 50, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.
32. The method of claim 31, wherein the amino acid sequence of the protein comprises:
 - a. at least 96.3% identity to an acyltransferase of clade 1 of Table 5;
 - b. at least 93.9% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 86.5% identity to an acyltransferase of clade 3 of Table 5; or

- d. at least 78.5% identity to an acyltransferase of clade 4 of Table 5.
33. The method of claim 31, wherein the amino acid sequence of the protein comprises:
- a. at least 98% identity to an acyltransferase of clade 1 of Table 5;
 - b. at least 98% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 90% identity to an acyltransferase of clade 3 of Table 5; or
 - d. at least 80% identity to an acyltransferase of clade 4 of Table 5.
34. The method of claim 31, wherein the amino acid sequence of the protein comprises:
- a. at least 99% identity to an acyltransferase of clade 16 of Table 5;
 - b. at least 99% identity to an acyltransferase of clade 2 of Table 5;
 - c. at least 95% identity to an acyltransferase of clade 3 of Table 5; or
 - d. at least 85% identity to an acyltransferase of clade 4 of Table 5.
35. The method of claim 31, wherein the amino acid sequence of the protein comprises:
- a. at least 98% identity to an acyltransferase of clade 3 of Table 5; or
 - b. at least 90% identity to an acyltransferase of clade 4 of Table 5.
36. The method of claim 31, wherein the amino acid sequence of the protein comprises:
- a. at least 99% identity to an acyltransferase of clade 3 of Table 5; or
 - b. at least 95% identity to an acyltransferase of clade 4 of Table 5.
37. The method of claim 31, wherein the amino acid sequence of the protein comprises at least 98% or 99% identity to an acyltransferase of clade 4 of Table 5.
38. The method of any of claims 31 to 37, wherein the cell is a microalgal cell, microbial cell or a plant cell.
39. The method of claim 38, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
40. The method of claim 39, wherein the cell is a *Prototheca moriformis* cell.
41. The method of any of claims 31 to 40, wherein the acyl transferase is a lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2).

42. The method of claim 41, wherein the acyl transferase is lysophosphatidic acid acyltransferase (LPAAT)
43. An oil composition produced by cultivating a host cell and recovering the oil composition from the host cell, the host cell comprising recombinant nucleic acids encoding a protein having acyltransferase activity, wherein the amino acid sequence of the acyltransferase has at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.
44. The oil composition of claim 43, wherein the amino acid sequence of the protein comprises:
- at least 96.3% identity to an acyltransferase of clade 1 of Table 5;
 - at least 93.9% identity to an acyltransferase of clade 2 of Table 5;
 - at least 86.5% identity to an acyltransferase of clade 3 of Table 5; or
 - at least 78.5% identity to an acyltransferase of clade 4 of Table 5.
45. The oil composition of claim 43, wherein the amino acid sequence of the protein comprises:
- at least 98% identity to an acyltransferase of clade 1 of Table 5;
 - at least 98% identity to an acyltransferase of clade 2 of Table 5;
 - at least 90% identity to an acyltransferase of clade 3 of Table 5; or
 - at least 80% identity to an acyltransferase of clade 4 of Table 5.
46. The oil of claim 43, wherein the amino acid sequence of the protein comprises:
- at least 99% identity to an acyltransferase of clade 1 of Table 5;
 - at least 99% identity to an acyltransferase of clade 2 of Table 5;
 - at least 95% identity to an acyltransferase of clade 3 of Table 5; or
 - at least 85% identity to an acyltransferase of clade 4 of Table 5.
47. The oil of claim 43, wherein the amino acid sequence of the protein comprises:
- at least 98% identity to an acyltransferase of clade 3 of Table 5; or
 - at least 90% identity to an acyltransferase of clade 4 of Table 5.
48. The oil 43, wherein the amino acid sequence of the protein comprises:
- at least 99% identity to an acyltransferase of clade 3 of Table 5; or
 - at least 95% identity to an acyltransferase of clade 4 of Table 5.

49. The oil 43, wherein the amino acid sequence of the protein comprises at least 98% or 99% identity to an acyltransferase of clade 4 of Table 5.
50. The oil composition of any of claims 43 to 49, wherein the cell is a microalgal cell, microbial cell or a plant cell.
51. The oil composition of claim 50, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
52. The oil composition of claim 51, wherein the cell is a *Prototheca moriformis* cell.
53. The oil composition of any of claims 43 to 52, wherein the acyl transferase is a lysophosphatidic acid acyltransferase (LPAAT), glycerol phosphate acyltransferase (GPAT), diacyl glycerol acyltransferase (DGAT), lysophosphatidylcholine acyltransferase (LPCAT), or phospholipase A2 (PLA2).
54. The oil composition of claim 53, wherein the acyltransferase is lysophosphatidic acid acyltransferase (LPAAT)
55. A recombinant protein having acyltransferase activity, wherein the amino acid sequence of the protein has at least 75%, 80%, 85%, 90%, 95%, 98% , 99%, or 100% identity to an acyltransferase of SEQ ID NOs: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 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, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196.
56. A recombinant vector construct or host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NO: 165, 166, 167, or 168 , the variant thioesterase comprising one or more of amino acid variants D124A, D209A, D127A or D212A.
57. The recombinant vector construct or host cell of claim 56, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
58. The recombinant vector construct or host cell of claim 56, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: 165, 166, 167, or 168.

59. The recombinant vector construct or host cell of any of claims 55 to 58, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.
60. The nucleic acids of claim 59, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
61. The nucleic acids of claim 59, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
62. A host cell expressing the recombinant protein any of claims 59 to 61, wherein the cell is a microalgal cell, microbial cell or a plant cell.
63. The host cell of claim 62, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
64. The host cell of claim 63, wherein the cell is a *Prototheca moriformis* cell.
65. A method of cultivating a host cell, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NO: 165, 166, 167, or 168, the variant thioesterase comprising amino acid variants D124A, D209A, or both D124A and D209A.
66. The method of claim 65, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
67. The method of claim 65, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
68. The nucleic acids of any of claims 65 to 67, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently

translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.

69. The nucleic acid of claim 68, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
70. The nucleic acid of claim 68, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
71. The method of any of claims 65 to 70, wherein the cell is a microalgal cell, microbial cell or a plant cell.
72. The method of claim 71, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
73. The method of claim 72, wherein the cell is a *Prototheca moriformis* cell.
74. A method of producing a triglyceride oil in a host cell, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NO: 165, 166, 167, or 168, the variant thioesterase comprising amino acid variants D124A, D209A, or both D124A and D209A, the method comprising the steps of:
 - a) providing the host cell;
 - b) adding a fixed carbon source to the culture medium; and
 - c) cultivating the host cell for a period of time.
75. The method of claim 74, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
76. The method of claim 74, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
77. The nucleic acids of any of claims 74 to 76, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.
78. The nucleic acid of claim 77, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons

of the coding sequence.

- 5 79. The nucleic acid of claim 77, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
80. The method of any of claims 74 to 79, wherein the cell is a microalgal cell, microbial cell or a plant cell.
- 10 81. The method of claim 80, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
82. The method of claim 81, wherein the cell is a *Prototheca moriformis* cell.
- 15 83. An oil composition produced by cultivating a host cell and recovering the oil composition from the host cell, the host cell comprising nucleic acids encoding a protein having an fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NO: 165, 166, 167, or 168, the variant
- 20 thioesterase comprising amino acid variants D124A, D209A, or both D124A and D209A, and wherein the oil composition comprises triglyceride oil and sterols produced by the host cell.
84. The oil composition of claim 83, wherein the nucleic acids encoding the protein
- 25 has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
85. The oil composition of claim 83, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
- 30 86. The oil of any of claims 83 to 85, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently
- 35 translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.
87. The oil of claim 86, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
- 40 88. The oil of claim 86, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the

coding sequence.

89. The oil composition of any of claims 83 to 88, wherein the cell is a microalgal cell, microbial cell or a plant cell.
90. The oil composition of cell of claim 89, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
91. The oil composition claim 90, wherein the cell is a *Prototheca moriformis* cell.
92. A recombinant vector construct or a host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NOs: 165, 166, 167, or 168, and wherein the variant thioesterase comprises amino acid variants D124A, D209A, or both D124A and D209A.
93. A recombinant vector construct or a host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150, the variant thioesterase comprising one or more amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V, T156F, T156A, T156K, T156V, or V193A.
94. The recombinant vector construct or a host cell of claim 92, wherein the amino acid sequence of the variant thioesterase has at least 90% identity to SEQ ID NOs: 165, 166, 167, or 168.
95. The recombinant vector construct or a host cell of claim 93, wherein the amino acid sequence of the variant thioesterase has at least 90% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
96. The recombinant vector construct or a host cell of claim 92, wherein the amino acid sequence of the variant thioesterase has at least 95% identity to SEQ ID NOs: 165, 166, 167, or 168.
97. The recombinant vector construct or a host cell of claim 93, wherein the amino acid sequence of the variant thioesterase has at least 95% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
98. The nucleic acids of any of claims 92 to 97, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca*

or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.

- 5 99. The nucleic acid of claim 98, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
- 10 100. The nucleic acid of claim 98, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
- 15 101. The host cell of any of claims 92 to 100, wherein the cell is a microalgal cell, microbial cell or a plant cell.
102. The host cell of claim 101, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
- 20 103. The host cell of claim 102, wherein the cell is a *Prototheca moriformis* cell.
104. A method of cultivating a host cell, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase
 - 25 has at least 80% or 85% identity to SEQ ID NOs: 165, 166, 167, or 168, and wherein the variant thioesterase comprises amino acid variants D124A, D209A, or both D124A and D209A.
105. A method of cultivating a host cell, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase
 - 30 has at least 80% or 85% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150, the variant thioesterase comprising one or more amino acid variants L91F, L91K, L91S, G96A, G96T, G96V,
 - 35 G108A, G108V, S111A, T156F, T156A, T156K, T156V, or V193A.
106. The method of claim 104, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
- 40 107. The method of claim 105, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or

108. The method of claim 104, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
109. The method of claim 105, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
110. The nucleic acids of any of claims 104 to 109, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.
111. The nucleic acid of claim 110, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
112. The nucleic acid of claim 110, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
113. The method of any of claims 104 to 112, wherein the cell is a microalgal cell, microbial cell or a plant cell.
114. The method of claim 113, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
115. The method of claim 114, wherein the cell is a *Prototheca moriformis* cell.
116. A method of producing a triglyceride oil, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase at least 80% or 85% identity to SEQ ID NOs: 165, 166, 167, or 168, and wherein the variant thioesterase comprises amino acid variants D124A, D209A, or both D124A and D209A, the method comprising the steps of: .
a) providing the host cell;
b) adding a fixed carbon source to the culture medium; and
c) cultivating the host cell for a period of time.
117. A method of producing a triglyceride oil, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein

- is a variant thioesterase and the amino acid sequence of the variant thioesterase at least 80% or 85% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150, the variant thioesterase comprising one or more amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V, T156F, T156A, T156K, T156V, or V193A, the method comprising the steps of: .
- a) providing the host cell;
 - b) adding a fixed carbon source to the culture medium; and
 - c) cultivating the host cell for a period of time.
118. The method of claim 116, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
119. The method of claim 117, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
120. The method of claim 116, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
121. The method of claim 117, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
122. The nucleic acids of any of claims 116 to 121, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.
123. The nucleic acid of claim 122, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
124. The nucleic acid of claim 122, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
125. The method of any of claims 116 to 124, wherein the cell is a microalgal cell, microbial cell or a plant cell.

126. The method of claim 125, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
127. The method of claim 126, wherein the cell is a *Prototheca moriformis* cell.
- 5 128. An oil composition produced by cultivating a host cell and recovering the oil composition from the host cell, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NOs: 165, 166, 167, or 168, and wherein the variant thioesterase comprises amino acid variants D124A, D209A, or both D124A and D209A.
- 10 129. An oil composition produced by cultivating a host cell and recovering the oil composition from the host cell, the host cell comprising nucleic acids encoding a protein having fatty acyl-ACP thioesterase activity, wherein the protein is a variant thioesterase and the amino acid sequence of the variant thioesterase has at least 80% or 85% identity to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150, the variant thioesterase comprising one or more amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V, T156F, T156A, T156K, T156V, or V193A, and wherein the oil composition comprises triglyceride oil and sterols produced by the host cell.
- 15 130. The oil composition of claim 128, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
- 20 131. The oil composition of claim 129, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
- 25 132. The oil composition of claim 128, wherein the nucleic acids encoding the protein has at least 90% identity to any of SEQ ID NOs: 165, 166, 167, or 168.
- 30 133. The oil composition of claim 129, wherein the nucleic acids encoding the protein has at least 95% identity to any of SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150.
- 35 134. The oil composition of any of claims 128 to 133, wherein the nucleic acids encoding the fatty acyl-ACP thioesterase are codon-optimized for expression in *Prototheca* or *Chlorella*, and wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 60% of the codons of the coding sequence, such that the codon-optimized sequence is more
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efficiently translated in *Prototheca* or *Chlorella* than a non-codon optimized sequence.

- 5 135. The oil composition of claim 134, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 80% of the codons of the coding sequence.
- 10 136. The oil composition of claim 134, wherein the coding sequence contains the most or second most preferred codon of Table 1 or Table 2 for at least 90% of the codons of the coding sequence.
137. The oil composition of any of claims 128 to 136, wherein the cell is a microalgal cell, microbial cell or a plant cell.
- 15 138. The oil composition of cell of claim 137, wherein the microalgal cell is a *Prototheca* cell or a *Chlorella* cell.
139. The oil composition claim 138, wherein the cell is a *Prototheca moriformis* cell.
- 20 140. A variant *Brassica napus* fatty acyl-ACP thioesterase having an amino acid sequence that has at least 80% or 85% identity to SEQ ID NOs: 165, 166, 167, or 168, and wherein the variant thioesterase comprises amino acid variants D124A, D209A, or both D124A and D209A.
- 25 141. A variant *Garcinia mangostana* fatty acyl-ACP thioesterase having an amino acid sequence that is at least 80% or 85% identical to SEQ ID NOs: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150, wherein the variant thioesterase comprises one or more amino acid variants L91F, L91K, L91S, G96A, G96T, G96V, G108A, G108V, S111A, S111V, T156F, T156A, 30 T156K, T156V, or V193A.

Fig.1

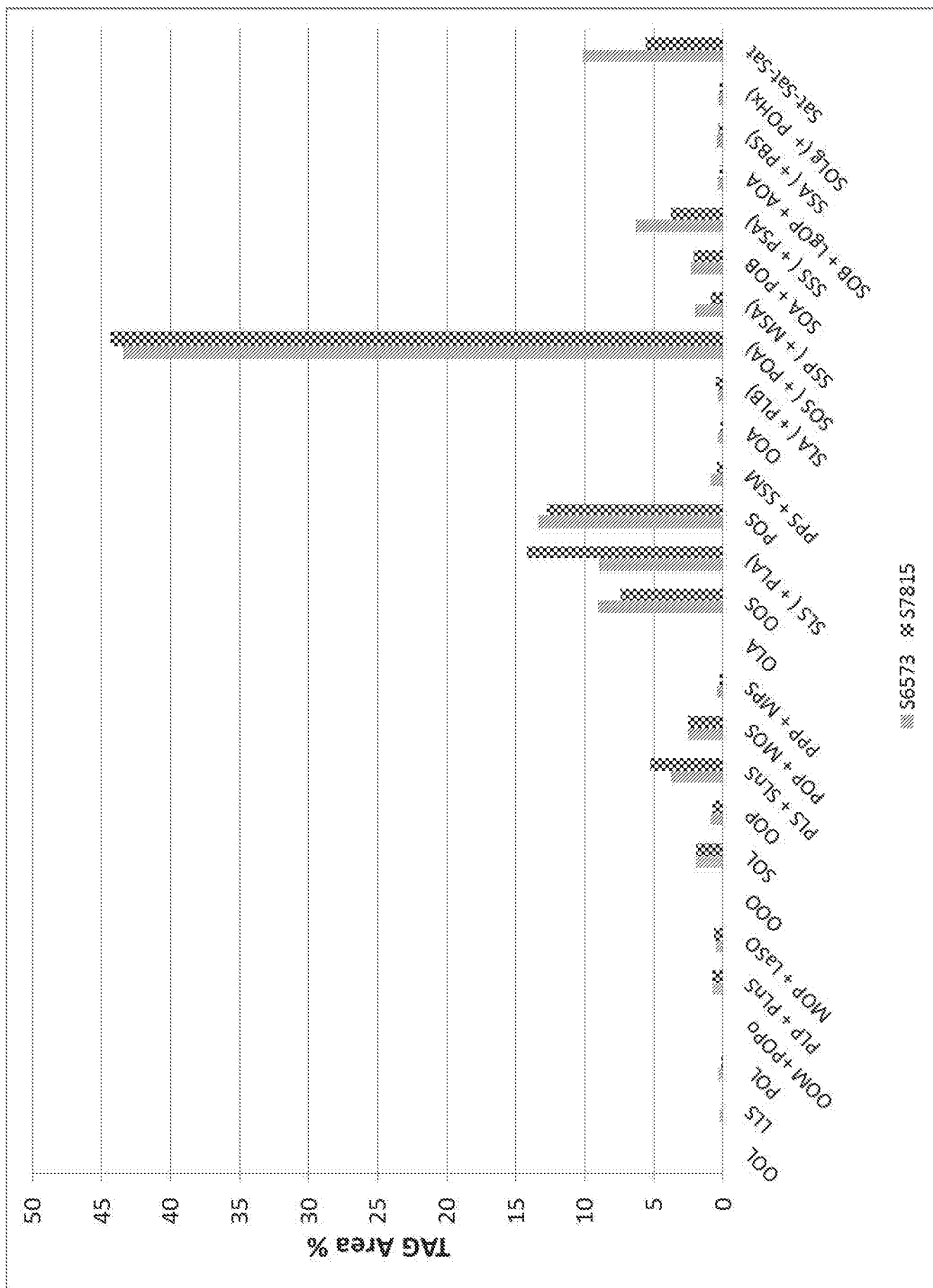


Fig.2

