

REGIOSPECIFIC TRIGLYCERIDE OILS AND BLENDS AND RELATED FOOD AND NUTRITION APPLICATIONS**BACKGROUND**

[0001] Oleaginous microorganisms have the ability to convert carbon substrates into oils, including triacylglycerides (TAGs) and lipids, and accumulate these oils intracellularly. TAGs are substantial components in food and nutrition. Human milk TAGs play an important role in infant nutrition and development. These TAGs present challenges in formulating infant foods and formulas that mimic the composition and functional properties of human milk. In particular, the TAG structure impacts the digestion, absorption, and bioavailability of lipophilic micronutrients and essential fatty acids that impact infant feeding and nutrition. The present invention is directed to providing a high oleate-palmitate-oleate oil for use in food and human nutrition applications.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This International Application claims the benefit of U.S. Provisional Application No. 63/595,229, filed on November 1, 2023.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing XML which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said Sequence Listing XML, created on October 30, 2024, is named 009919_00093_WO_SL.xml and is 535,010 bytes in size.

INCORPORATION BY REFERENCE

[0004] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Various features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0006] **FIG. 1** illustrates two TAG species of the same molecular weight showing distinctive daughter ion ratios. **Panel A** shows OOP and **Panel B** shows OPO.

[0007] **FIG. 2** illustrates the mass spectra of OOP/OPO triglycerides from oil produced from various strains of microalgae provided herein. **Panel A** shows CHK22, D552-1; **Panel B** shows CHK22, D552-3; **Panel C** shows CHK22, D552-4; **Panel D** shows the non-transgenic strain CHK22.

[0008] **FIG. 3, Panels A-C** illustrate amino acid sequence alignments of LPAATs described herein. EcPlsC (SEQ ID NO: 22), OilLPAAT2 (SEQ ID NO: 63), PmLPAAT2 (SEQ ID NO: 24), CosLPAAT2 (SEQ ID NO: 64), CrLPAAT2 (SEQ ID NO: 13), ChsLPAAT2 (SEQ ID NO: 14), CilLPAAT2 (SEQ ID NO: 15), ValLPAAT2 (SEQ ID NO: 21), VcLPAAT2 (SEQ ID NO: 20), NoLPAT4 (SEQ ID NO: 17), NoLPAT3 (SEQ ID NO: 16), PmLPAAT1 (SEQ ID NO: 23), Sll1848 (SEQ ID NO: 19), BnBAT2 (SEQ ID NO: 18), CrLPAAT1 (SEQ ID NO: 12), and consensus (SEQ ID NO: 157).

[0009] **FIG. 4** illustrates schematic diagrams showing the structural domains of LPAATs described herein.

[0010] **FIG. 5** is a bar graph showing the fatty acid composition at the *sn*-2 position for oils from various strains, including strains that have been transformed with pCHK385.

[0011] **FIG. 6** illustrates LC-MS results for oil from CHK22 and strain D552-3.

[0012] **FIG. 7** illustrates TAG oils enriched in OPO and downstream TAG intermediates generated through chemical conversion processes.

[0013] **FIG. 8** illustrates an example diol produced from a TAG oil enriched in OPO.

[0014] **FIG. 9** illustrates an overview of an example classical strain improvement strategy for the generation of mutants derived from CHK22 (UTEX 1533).

[0015] **FIG. 10** illustrates the strain improvement strategy of CHK22 to produce CHK100.

[0016] **FIG. 11** illustrates the transmembrane domains of LPAATs disclosed herein.

[0017] **FIG. 12** illustrates the health benefits of *sn*-2 palmitate in infants, reproduced from Havlicekova et al. 2015.

[0018] **FIG. 13** compares the LC-MS triacylglycerol TAG profiles of the algae OPO oil of an aspect of the present disclosure and first generation of human milk fat substitute obtained by enzymatically catalyzed transesterification of vegetable oils.

SUMMARY

[0019] In some embodiments, provided herein is a non-naturally occurring oil comprising: at least 10 mg of ergosterol per 100 g of the oil; and a triacylglyceride (TAG) component, wherein

at least 40% of TAGs in the TAG component are TAG species having a saturated fatty acid at the sn-2 position, wherein at least 50% of the acyl chains in the TAG component are C18:1. The saturated fatty acid can be C16:0. At least 50% of the TAGs in the TAG component can be TAG species having a saturated fatty acid at the sn-2 position. At least 50% of the acyl chains in the TAG component can be C18:1. The TAG species can comprise C18:1 at the sn-1 and sn-3 positions. The TAG species can comprise C16:0 at the sn-2 position. The TAG species can comprise or consist of 1,3-dioleoyl-2-palmitoyl glycerol (OPO). At least 50% of the TAGs in the TAG component can be C18:1 and at least 20% of the acyl chains in the TAG component can be C16:0. At least 60% of the acyl chains in the TAG component can be C18:1 and at least 30% of the acyl chains in the TAG component can be C16:0. In some embodiments 50-67% or 60-67% of the acyl chains in the TAG component can be C18:1 and 20-33% of the acyl chains in the TAG component can be C16:0. The oil can have an OP:OO m/z ratio of at least 1.6 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 2 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 3 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 4 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can comprise at least 50 mg of ergosterol per 100 g of the oil. The oil can comprise at least 100 mg of ergosterol per 100 g of the oil. The oil can comprise 100-200 mg of ergosterol per 100 g of the oil. The oil can further comprise no more than 5 mg of campesterol, no more than 5 mg of β -sitosterol, or no more than 5 mg of stigmasterol per 100 g of the oil. For example, the oil can comprise no more than 5 mg of campesterol per 100 g of the oil. In some embodiments, the oil does not comprise campesterol. For example, the oil can comprise no more than 5 mg of stigmasterol per 100 g of the oil. In some embodiments, the oil does not comprise stigmasterol. For example, the oil can comprise no more than 5 mg of β -sitosterol per 100 g of the oil. In some embodiments, the oil does not comprise β -sitosterol. The oil can further comprise one or more of ergosta-5,9-dien-3-ol, (3 β)-, 5.xi.-ergost-7-en-3 β -ol, 9,19-cyclolanostan-3-ol, 24-methylene-, (3 β)-, and ergosta-7,22-dien-3-ol, (3 β). The oil can comprise at least 1 mg of ergosta-5,9-dien-3-ol, (3 β)- per 100 g of the oil. The oil can comprise 1-50 mg of ergosta-5,9-dien-3-ol, (3 β)- per 100 g of the oil. The oil can comprise at least 15 mg of 5.xi.-ergost-7-en-3 β -ol per 100 g of the oil. The oil can comprise 1-50 mg of 5.xi.-ergost-7-en-3 β -ol per 100 g of the oil. The oil can comprise at

least 20 mg of 5.xi.-ergost-7-en-3 β -ol per 100 g of the oil. The oil can comprise at least 5 mg of 9,19-cyclolanostan-3-ol,24-methylene-, (3 β)- per 100 g of the oil. The oil can be an algal oil. The oil can be a genetically modified algal oil. The oil can be produced from an algal cell. The oil can be produced from a genetically modified algal cell. The oil can be produced from a *Prototheca* cell.

[0020] Disclosed herein is a composition comprising the oil and one or more excipients. The composition can be a nutritional supplement. The composition can be an infant formula. The infant formula can comprise one or more of whey, casein, lactose, vitamin D, human milk oligosaccharides (HMOs), vegetable oils, and antibodies. Non-limiting examples of vegetable oils include soy oil, canola oil, sunflower oil, coconut oil, palm oil, and palm kernel oil. The infant formula can comprise soy protein. Disclosed herein is a TAG polyol produced from the oil.

[0021] Disclosed herein is a method for producing a TAG polyol, the method comprising: subjecting an oil described herein to epoxidation, thereby generating an epoxidized oil; and ring opening the epoxidized oil in the presence of an alcohol, an acid, or hydrogen and a suitable catalyst, thereby generating the TAG polyol. The epoxidized oil can be ring opened in the presence of an alcohol. The epoxidized oil can be ring opened in the presence of hydrogen and a suitable catalyst. The epoxidized oil can be ring opened in the presence of the acid. Disclosed herein is a TAG polyol produced by a method described herein.

[0022] Disclosed herein is a method for producing a TAG polyol, the method comprising: subjecting an oil described herein to hydroformylation, thereby generating a hydroformylated oil; and reducing the hydroformylated oil in the presence of hydrogen and a suitable catalyst, thereby generating the TAG polyol. Disclosed herein is a TAG polyol produced by a method described herein.

[0023] Disclosed herein is a microalgal cell comprising an exogenous gene that encodes for an enzyme having lysophosphatidic acid acyltransferase activity, wherein the cell produces an oil comprising a TAG component, wherein at least 40% of TAGs in the TAG component can be TAG species having a saturated fatty acid at the sn-2 position, wherein at least 50% of the acyl chains in the TAG component can be C18:1. The enzyme can be a lysophosphatidic acid acyltransferase (LPAAT). The enzyme can be an algal, higher plant or mammalian lysophosphatidic acid acyltransferase (LPAAT).

[0024] In some embodiments, an enzyme disclosed herein can comprise a sequence motif selected from any one of (I) NHXXXXD (or NHX₄D); (II) (F/Y)XXR; (III) EGXR; and (IV) Proline, wherein X is any amino acid.

[0025] In some embodiments, the enzyme can comprise NHXXXXD (or NHX₄D), wherein X is any amino acid. In some embodiments, the enzyme can comprise a sequence with at least 70%, at least 85%, or 100% sequence identity to any one of SEQ ID NO: 107-109.

[0026] In some embodiments, the enzyme can comprise (F/Y)XXR, wherein X is any amino acid. In some embodiments, the enzyme can comprise a sequence with at least 60%, at least 75%, at least 85%, or 100% sequence identity to any one of SEQ ID NO: 110-119.

[0027] In some embodiments, the enzyme can comprise EGXR, wherein X is any amino acid. In some embodiments, the enzyme can comprise a sequence with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or 100% sequence identity to any one of SEQ ID NO: 120-134.

[0028] In some embodiments, the enzyme can comprise a sequence a sequence with a conserved proline. In some embodiments, the enzyme can comprise a sequence with at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or 100% sequence identity to any one of SEQ ID NO: 135-154.

[0029] The enzyme can be *Chlamydomonas reinhardtii* lysophosphatidic acid acyltransferase (CrLPAAT1). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2.

[0030] The enzyme can be *Chlamydomonas incerta* lysophosphatidic acid acyltransferase (CiLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10.

[0031] The enzyme can be *Chlamydomonas schloesseri* lysophosphatidic acid acyltransferase (ChsLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 9.

[0032] The enzyme can be *Volvox africanus* lysophosphatidic acid acyltransferase (VaLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 21. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11.

[0033] The enzyme can be *Nannochloropsis oceanica* lysophosphatidic acid acyltransferase (NoLPAT3). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 7.

[0034] The enzyme can be *Synechocystis sp.* 1-acyl-sn-glycerol-3-phosphate acyltransferase (Sl11848). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 19. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 5.

[0035] The enzyme can be *Chlamydomonas reinhardtii* lysophosphatidic acid acyltransferase (CrLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1.

[0036] The enzyme can be *Escherichia coli* 1-acyl-sn-glycerol-3-phosphate acyltransferase (EcPlsC). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 22. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6.

[0037] The enzyme can be *Volvox carteri* lysophosphatidic acid acyltransferase (VcLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%

sequence identity to SEQ ID NO: 20. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8. The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 40. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25.

[0038] The enzyme can be *Brassica napus* acyltransferase (BnBAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3. The enzyme can be *Nannochloropsis oceanica* lysophosphatidic acid acyltransferase (NoLPAT4). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

[0039] The enzyme can be *Astrephomene gubernaculifera* lysophosphatidic acid acyltransferase (AgLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 41. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26.

[0040] The enzyme can be *Edaphochlamys debaryana* lysophosphatidic acid acyltransferase (EdLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27.

[0041] The enzyme can be *Dunaliella salina* lysophosphatidic acid acyltransferase (DsLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%

sequence identity to SEQ ID NO: 43. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28.

[0042] The enzyme can be *Scenedesmus sp.* lysophosphatidic acid acyltransferase (SceLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 44. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 29.

[0043] The enzyme can be *Micractinium conductrix* lysophosphatidic acid acyltransferase (McLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 45. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30.

[0044] The enzyme can be *Chlorella sorokiniana* lysophosphatidic acid acyltransferase (ChsoLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 46. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31.

[0045] The enzyme can be *Chlorella variabilis* lysophosphatidic acid acyltransferase (ChvaLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 47. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 32.

[0046] The enzyme can be *Raphidocelis subcapitata* lysophosphatidic acid acyltransferase (RsLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 48. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 33.

[0047] The enzyme can be *Chlorella desiccata* lysophosphatidic acid acyltransferase (ChdeLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 49. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 34.

[0048] The enzyme can be *Auxenochlorella protothecoides* lysophosphatidic acid acyltransferase (ApLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 50. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 35.

[0049] The enzyme can be *Chloropicon primus* lysophosphatidic acid acyltransferase (ChprPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 51. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36.

[0050] The enzyme can be *Homo sapiens* lysophosphatidic acid acyltransferase (AGPAT1). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 52. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 37.

[0051] The enzyme can be *Chlamydomonas eustigma* lysophosphatidic acid acyltransferase (CeLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 53. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 38.

[0052] The enzyme can be a Pedinophyceae lysophosphatidic acid acyltransferase (PedLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%

sequence identity to SEQ ID NO: 54. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 39. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 59. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 50. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 61. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 62.

[0053] The enzyme can be *Volvox reticuliferus* lysophosphatidic acid acyltransferase (VrLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 55. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 57. The enzyme can be *Chlorella vulgaris* lysophosphatidic acid acyltransferase (ChvuLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 56. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 58.

[0054] The enzyme can be *Volvulina compacta* lysophosphatidic acid acyltransferase (VcomLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 83. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 65.

[0055] The enzyme can be *Vitreochlamys sp. CL-2021* lysophosphatidic acid acyltransferase (VitrLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 84. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 66.

[0056] The enzyme can be *Colemanosphaera charkowiensis* lysophosphatidic acid acyltransferase (CchaLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 85. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 67.

[0057] The enzyme can be *Pleodorina japonica* lysophosphatidic acid acyltransferase (PjapLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 86. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 68.

[0058] The enzyme can be *Volvulina boldii* lysophosphatidic acid acyltransferase (VbolLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 87. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 69.

[0059] The enzyme can be *Pandorina morum* lysophosphatidic acid acyltransferase (PmorLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 88. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 70.

[0060] The enzyme can be *Volvox carteri* f. *weismannia* lysophosphatidic acid acyltransferase (VcarfLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 89. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 71.

[0061] The enzyme can be *Eudorina cylindrica* lysophosphatidic acid acyltransferase (EcyLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least

99%, or 100% sequence identity to SEQ ID NO: 90. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 72.

[0062] The enzyme can be *Gonium multicoccum* lysophosphatidic acid acyltransferase (GmulLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 91. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 73.

[0063] The enzyme can be *Gonium viridistellatum* lysophosphatidic acid acyltransferase (GvirLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 92. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 74.

[0064] The enzyme can be *Volvox ferrisii* lysophosphatidic acid acyltransferase (VferLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 93. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 75.

[0065] The enzyme can be *Vitreochlamys aulata* lysophosphatidic acid acyltransferase (VaulLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 94. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 76.

[0066] The enzyme can be *Chlamydomonas sp. CCAC2762_B* lysophosphatidic acid acyltransferase (Ch_CCAC2762_LPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 95. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 77.

[0067] The enzyme can be *Dunaliella salina* lysophosphatidic acid acyltransferase (DsalLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 96. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 78.

[0068] The enzyme can be *Microglena sp.* YARC lysophosphatidic acid acyltransferase (MyarcLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 97. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 79.

[0069] The enzyme can be *Chlamydomonas sp.* UWO_241 lysophosphatidic acid acyltransferase (CuwoLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 98. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 80.

[0070] The enzyme can be *Chlamydomonas moewusii* lysophosphatidic acid acyltransferase (CmoeLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 99. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 81.

[0071] The enzyme can be *Oophila amblystomatis* lysophosphatidic acid acyltransferase (OambLPAAT2). The enzyme can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 100. The cell can comprise a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 82.

[0072] The exogenous gene can be codon-optimized for expression in a *Prototheca* strain. In some embodiments, the cell does not comprise an exogenous glycerol-3-phosphate acyltransferase (GPAT1). The cell can be from a *Prototheca* base strain. The cell can be from the

Prototheca moriformis base strain UTEX 1533. The cell can be derived from a classically improved strain from *Prototheca moriformis* base strain UTEX 1533. The cell can be from a non-genetically modified *Prototheca* base strain that produces an oil having a fatty acid profile of at least 50% oleic acid. The cell can be from a non-genetically modified *Prototheca* base strain that produces an oil having a fatty acid profile of at least 30% palmitic acid. The *Prototheca* base strain can be *Prototheca wickerhamii*. The *Prototheca* base strain can be *Prototheca moriformis*. The microalgal cell can produce at least 60% lipid by dry cell weight. The microalgal cell can produce at least 50% lipid by dry cell weight. The oil can comprise a triacylglyceride (TAG) component, wherein at least 40% of TAGs in the TAG component can be TAG species having a saturated fatty acid at the sn-2 position, wherein at least 50% of the acyl chains in the TAG component can be C18:1. The oil can comprise at least 10 mg of ergosterol per 100 g of the oil. The saturated fatty acid can be C16:0. At least 50% of the TAGs in the TAG component can be TAG species having a saturated fatty acid at the sn-2 position. At least 50% of the acyl chains in the TAG component can be C18:1. The TAG species can comprise C18:1 at the sn-1 and sn-3 positions. The TAG species can comprise C16:0 at the sn-2 position. The TAG species can comprise or consist of 1,3-dioleoyl-2-palmitoyl glycerol (OPO). At least 50% of the acyl chains in the TAG component can be C18:1 and at least 20% of the acyl chains in the TAG component can be C16:0. At least 60% of the acyl chains in the TAG component can be C18:1 and at least 30% of the acyl chains in the TAG component can be C16:0. In some embodiments 50-67% or 60-67% of the acyl chains in the TAG component can be C18:1 and 20-33% of the acyl chains in the TAG component can be C16:0. The oil can have an OP:OO m/z ratio of at least 1.6 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 2 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 3 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can comprise at least 50 mg of ergosterol per 100 g of the oil. The oil can comprise at least 100 mg of ergosterol per 100 g of the oil. The oil can further comprise one or more of 5.xi.-ergost-7-en-3 β -ol, 9,19-cyclolanostan-3-ol, 24-methylene-, (3 β)-, and ergosta-7,22-dien-3-ol, (3 β). The oil can comprise at least 20 mg of 5.xi.-ergost-7-en-3 β -ol per 100 g of the oil. The oil can comprise at least 5 mg of 9,19-cyclolanostan-3-ol, 24-methylene-, (3 β)- per 100 g of the oil. The oil can be an algal oil. The oil can be a genetically modified algal oil. The oil can

be produced from an algal cell. The oil can be produced from a genetically modified algal cell. The oil can be produced from a *Prototheca* cell.

[0073] Disclosed herein is a method of producing a non-naturally occurring oil, the method comprising cultivating a microalgal cell in a culture medium, wherein the oil comprises a TAG component, wherein at least 40% of TAG species in the TAG component have a saturated fatty acid at the sn-2 position, wherein at least 50% of the acyl chains in the TAG component can be C18:1. The method can further comprise isolating the oil composition from the culture medium. The method can further comprise expressing in the microalgal cell an exogenous enzyme having lysophosphatidic acid acyltransferase activity. The microalgal cell can be a microalgal cell described herein. The oil can comprise a triacylglyceride (TAG) component, wherein at least 40% of the TAGs in the TAG component can be TAG species having a saturated fatty acid at the sn-2 position, wherein at least 50% of the acyl chains in the TAG component can be C18:1. The oil can comprise at least 10 mg of ergosterol per 100 g of the oil. The saturated fatty acid can be C16:0. At least 50% of the TAGs in the TAG component can be TAG species having a saturated fatty acid at the sn-2 position. At least 50% of the acyl chains in the TAG component can be C18:1. The TAG species can comprise C18:1 at the sn-1 and sn-3 positions. The TAG species can comprise C16:0 at the sn-2 position. The TAG species can comprise or consist of 1,3-dioleoyl-2-palmitoyl glycerol (OPO). At least 50% of the acyl chains in the TAG component can be C18:1 and at least 20% of the acyl chains in the TAG component can be C16:0. At least 60% of the acyl chains in the TAG component can be C18:1 and at least 30% of the acyl chains in the TAG component can be C16:0. 50-67% or 60-67% of the acyl chains in the TAG component can be C18:1 and 20-33% of the acyl chains in the TAG component can be C16:0. The oil can have an OP:OO m/z ratio of at least 1.6 as determined by abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 2 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can have an OP:OO m/z ratio of at least 3 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. The oil can comprise at least 50 mg of ergosterol per 100 g of the oil. The oil can comprise at least 100 mg of ergosterol per 100 g of the oil. The oil can further comprise one or more of 5.xi.-ergost-7-en-3 β -ol, 9,19-cyclolanostan-3-ol, 24-methylene-, (3 β)-, and ergosta-7,22-dien-3-ol, (3 β). The oil can comprise at least 20 mg of 5.xi.-ergost-7-en-3 β -ol per 100 g of the oil. The oil can comprise at least 5 mg of 9,19-cyclolanostan-3-ol, 24-methylene-, (3 β)- per 100 g of the oil. The oil can be an algal oil. The oil can be a genetically modified algal

oil. The oil can be produced from an algal cell. The oil can be produced from a genetically modified algal cell. The oil can be produced from a *Prototheca* cell.

[0074] Traditionally, microalgae have been known for producing long-chain polyunsaturated fatty acids (LC-PUFAs) like docosahexaenoic (DHA) or eicosapentaenoic (EPA) acids, commonly used in infant nutrition, supplements, and foods for humans and animals. There is a need in the industry to optimize the fatty acid composition and triacylglycerol structure of the oil produced by microalgae; thereby expanding the potential applications of microalgae oil beyond LC-PUFAs, tailoring them for specific nutritional or physiochemical properties. Specifically, aspects of the instant application contain oil derived from microalgae which is enriched with saturated fatty acid at the sn-2 position, and in particular, palmitate.

[0075] In some embodiments, provided herein is a non-naturally occurring oil comprising: at least 10 mg of ergosterol per 100 g of the oil; and a triacylglyceride (TAG) component, wherein at least 40% of TAGs in the TAG component are TAG species having a saturated fatty acid at the sn-2 position, wherein at least 50% of the acyl chains in the TAG component are C18:1. The oil can be an algal oil. The oil can be a genetically modified algal oil. The oil can be produced from an algal cell. The oil can be produced from a genetically modified algal cell. The oil can be produced from a *Prototheca* cell.

[0076] Disclosed herein is a composition comprising the oil and one or more excipients. The composition can be a food product. The composition can be a food or beverage. Further, the composition can be a nutritional supplement. More specifically, the composition can be an infant formula.

[0077] Disclosed herein is a composition comprising the oil and one or more excipients. The composition can be a nutritional supplement. The composition can be an infant formula. The infant formula can comprise one or more of whey, casein, lactose, vitamin D, human milk oligosaccharides (HMOs), vegetable oils, and antibodies. Non-limiting examples of vegetable oils include soy oil, canola oil, sunflower oil, coconut oil, palm oil, and palm kernel oil. The infant formula can comprise soy protein.

[0078] The oil can be an algal oil. The oil can be a genetically modified algal oil. The oil can be produced from an algal cell. The oil can be produced from a genetically modified algal cell. The oil can be produced from a *Prototheca* cell.

DETAILED DESCRIPTION

[0079] Provided herein are oil compositions comprising TAGs enriched in 1,3-dioleoyl-2-palmitoylglycerol (OPO), methods of making, formulations, and applications thereof. Oil

compositions provided herein can be produced by a genetically modified microorganism provided herein, such as a microalgal cell provided herein. Further provided are methods of producing and cultivating microorganisms that produce the oil compositions provided herein. Further provided herein are bioreactors comprising a non-naturally occurring microorganism provided herein that has been modified to produce the oil compositions provided herein.

Definitions

[0080] As used herein, the term “microbial oil” refers to an oil produced or extracted from a microorganism (microbe), e.g., an oleaginous, single-celled, eukaryotic, or prokaryotic microorganism, including but not limited to, microalgae, yeast, bacteria, and fungi.

[0081] As used herein, the term “triacylglycerol”, “triglyceride”, or “TAG” refers to esters between glycerol and three fatty acids.

[0082] As used herein, the term “OPO” or “1,3-dioleoyl-2-palmitoylglycerol” refers to a TAG species having a palmitate (C16:0) at the *sn*-2 position of the TAG and oleate (C18:1) at the *sn*-1 and *sn*-3 positions of the TAG.

[0083] As used herein, the term “OOP” or “1,2-dioleoyl-3-palmitoylglycerol” refers to a TAG species having a palmitate (C16:0) at the *sn*-3 position of the TAG and oleate (C18:1) at the *sn*-1 and *sn*-2 positions of the TAG.

[0084] As used herein, the term “polyol” refers to triglycerols or fatty acid alcohols comprising hydroxyl functional groups. As used herein, the term “polyol derived from a TAG oil” generally refers to a polyol obtained from chemical conversion of a TAG oil, e.g., via epoxidation and ring opening, ozonolysis, and reduction, or hydroformylation and reduction.

[0085] As used herein, the term “polyurethane”, “PU”, or “urethane” refers to a class of polymers comprised of carbamate (urethane) linkages formed between a polyol and an isocyanate moiety.

[0086] As used herein, the term “high oleic” can refer to greater than 60% oleic acid, greater than 70% oleic acid, greater than 80% oleic acid, or greater than 90% oleic acid.

[0087] As used herein, the term “about” refers to and can be $\pm 10\%$ of the value provided.

[0088] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present teachings, some exemplary methods and materials are described herein.

Human Breast Milk

[0089] Human breast milk is the primary source of nutrition for newborn babies. Breast milk is comprised of fat, protein, carbohydrates, and various vitamins and minerals necessary to support healthy growth and development. Breast milk also contains factors that are important for protecting the infant against infection and inflammation, while also contributing to healthy development of the immune system and gut microbiome.

[0090] Breast milk contains about 3-55% fat, and 98% of which is in the form of TAGs containing more than 200 types of different fatty acids. Breast milk contains about 17-25% palmitic acid and 70-75% of which is uniquely distributed in the stereotypically numbered 2 (*sn*-2) position of TAGs, while unsaturated fatty acids, such as oleic acid and linoleic acid, are mostly distributed in the *sn*-1 and *sn*-3 positions. The high content of palmitic acid in the *sn*-2 position may contribute to enhanced absorption of calcium and essential fats in the infant gut, and overall digestive health. A key TAG in human breast milk is 1,3-dioleoyl-2-palmitoylglycerol, also referred to as *sn*-2 palmitate, oleic-palmitic-oleic, or OPO. OPO is found in human breast milk, but is generally absent in bovine milk.

[0091] Given the complex nature of human breast milk, infant formula manufacturers have struggled to recapitulate even a fraction of the features present in this remarkable, natural food. Some attention has been paid to achieving the relative proportions of desired fatty acids in infant formulas to recapitulate the components of breast milk. Products have also been created that recapitulate TAGs enriched in OPO that can be combined with other fat fractions to mimic human breast milk. Human milk fat substitute (HMFS) is a class of structured lipids that is widely used as an ingredient in infant formulas. Like human milk fat, HMFS is characterized by the enrichment of palmitoyl (C16:0) groups specifically at the middle (*sn*-2 or β) position on the glycerol backbone. However, no single-source fat substitute delivers all the demonstrated benefits of the natural product.

[0092] Although the fat used in most infant formulas is derived from plants, many plants esterify saturated fatty acids to the *sn*-1 and *sn*-3 positions, and exclude palmitic acid in the *sn*-2 position. In contrast, engineered oilseed crops and/or oleaginous microorganisms can be used to accumulate high levels of TAGs enriched in saturated fatty acid in the *sn*-2 position, such as OPO. These alternative sources of TAGs may thereby better mimic the stereoisomeric structure of human milk fat and provide improved sources of HMFS for infant nutrition.

[0093] Infant formulas produced with vegetable oils or dairy products have similar fat content to fatty acids in human milk while presenting different lipid structures. For instance, while about

70% of palmitic acid is esterified at the *sn*-2 position of the TAGs in human milk; in comparison, vegetable oils and other mammalian (e.g., cow, sheep, and goat) milk have only about 5-20% and about 30-40% respectively, of the total palmitic acid esterified at the *sn*-2 position. As a result, the lipid components used in most infant formulas originating from plants or derived from dairy products have the majority of palmitic acid esterified at the *sn*-1/3 positions.

[0094] Human milk fat substitutes are a class of structured lipids developed to mimic the TAG distribution of human breast milk and obtained by altering the composition, structure and distribution of fatty acids attached to the glycerol backbone. The inventors of the instant application found that the oils disclosed in this application, including the TAG oils derived from microalgae enriched in OPO, can be used as a human milk fat substitute composed of structured TAGs enriched in palmitic acid at the *sn*-2 position. The strain may be derived from classical strain improvement or genetic modification.

[0095] In one aspect, the algal-derived OPO enriched TAG oil is manufactured from microalgae derived from classical strain improvement. In another aspect, the algal-derived OPO enriched TAG oils are manufactured from a genetically-modified microalgae. The microalgae strain may be selected from, without limitation, *P. moriformis*. The OPO oil may be included as a partial replacement for vegetable oils in conventional infant nutrition products such as infant formulas, follow-on formulas and pediatric supplements intended for infants born at term or for premature infants. For example, Figure 13 compares the LC-MS triacylglycerol TAG profiles of the algae OPO oil of an aspect of the present disclosure and first generation of human milk fat substitute obtained by enzymatically catalyzed transesterification of vegetable oils. As can be seen from Figure 13, the amount of OPO in the OPO oil of the present disclosure significantly exceeds that of the commercially available vegetable derived alternatives.

[0096] The natural occurrence of palmitic acid in the *sn*-2 position of triglycerides is associated with crucial biological functions in infants, as depicted in Figure 12 (adapted from Havlicekova et al., 2016, Beta-palmitate - a natural component of human milk in supplemental milk formulas. Nutr J. 15:28. doi: 10.1186/s12937-016-0145-1). Human milk triglycerides are intrinsically structured to optimize the absorption of fatty acids, primarily due to the presence of palmitic acid in the *sn*-2 position. Achieving quantitative absorption of essential milk nutrients is of paramount importance for the healthy development of infants, as lipids constitute the primary source of energy during infancy and play pivotal roles in various developmental processes. Moreover, the absorption of critical micronutrients, such as calcium, is intricately linked to the absorption of palmitic acid.

[0097] When triglycerides provided to infants contain palmitic acid esterified in the external positions of the triglyceride molecules, the liberated palmitic acid, as a consequence of lipolytic enzyme action, tends to form insoluble salts through its interaction with calcium. The accumulation of these insoluble salts within the infant's gastrointestinal tract (GI tract) can significantly influence the development of the microbiome and result in discomfort for the infant, characterized by the formation of firmer stools. This disruption in microbiome development caused by an excessive presence of insoluble palmitic acid salts within the GI tract can potentially trigger inflammation and immune-related issues. By formulating infant nutrition products with an oil source that contains palmitic acid in the *sn*-2 position, such as algae-derived OPO oil, it is feasible to replicate the nutritional advantages associated with naturally occurring *sn*-2 palmitate in human milk when compared to conventional alternatives.

TAG Biosynthesis

[0098] Lysophosphatidic acid acyltransferase (LPAAT), a type of membrane-bound o-acyltransferase (MBOAT) encoded by a multigene family, is a rate-limiting enzyme in the Kennedy pathway in higher plants. The Kennedy pathway turns glycerol-3-phosphate into TAG using glycerol-3-phosphate acyltransferase (GPAT), LPAAT, phosphatidic acid phosphatase (PAP), and diacylglycerol acyltransferase (DGAT). In this pathway, LPAAT controls the production of phosphatidic acid (PA), a key intermediate.

[0099] LPAAT genes are present in both prokaryotes and eukaryotes. In mammals, LPAATs have been characterized as the 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT) family. For example, a *Homo sapiens* AGPAT gene is *AGPAT1*. *AGPAT1* localizes to the endoplasmic reticulum. In some embodiments, the amino acid sequence of *AGPAT1* is SEQ ID NO: 52. In some embodiments, *AGPAT1* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 37. Plants also often have several LPAAT genes; for example, *Arabidopsis thaliana* has 5 LPAAT genes. In most microalgae and higher plants, TAG biosynthesis occurs both in the chloroplast and endoplasmic reticulum (ER), and the substrate preference for LPAAT enzymes is usually oleoyl-CoA. Among other eukaryotes, however, including animals and fungi, the main organelle for TAG biosynthesis is the endoplasmic reticulum (ER), and once again, the substrate preference for LPAAT enzymes is usually oleoyl-CoA.

[0100] The enzyme having lysophosphatidic acid acyltransferase activity can comprise four catalytic motifs: (I) NHXXXXD (or NHX₄D); (II) (F/Y)XXR; (III) EGXR; and (IV) Proline,

wherein X is any amino acid. Motif I can comprise a conserved NHXXXXD (or NHX₄D) sequence in which the residue following histidine (H) is usually hydrophilic, whereas the residue preceding aspartic acid (D) is almost always hydrophobic. Motif II can comprise a (F/Y)XXR sequence, in which the first residue is either phenylalanine (F) or tyrosine (Y) followed by two residues and a conserved arginine (R). The residue that precedes this R can be hydrophilic, whereas the residue that precedes this hydrophilic residue can be hydrophobic. Motif III can comprise a conserved EGXR sequence. Motif IV can comprise a conserved proline (P). An enzyme described herein can comprise a sequence with one, two, three, or four of these catalytic motifs. A cell described herein can comprise an exogenous gene comprising a sequence that encodes for an enzyme with one, two, three, or four of these catalytic motifs.

[0101] In some embodiments, an enzyme can comprise a sequence with at least 70%, at least 85%, or 100% sequence identity to any one of SEQ ID NO: 107-109. In some embodiments, a cell can comprise an exogenous gene comprising a sequence that encodes for an enzyme with at least 70%, at least 85%, or 100% sequence identity to any one of SEQ ID NO: 107-109.

[0102] In some embodiments, an enzyme can comprise a sequence with at least 60%, at least 75%, at least 85%, or 100% sequence identity to any one of SEQ ID NO: 110-119. In some embodiments, a cell can comprise an exogenous gene comprising a sequence that encodes for an enzyme with at least 60%, at least 75%, at least 85%, or 100% sequence identity to any one of SEQ ID NO: 110-119.

[0103] In some embodiments, an enzyme can comprise EGHR or EGTR. In some embodiments, an enzyme can comprise a sequence with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or 100% sequence identity to any one of SEQ ID NO: 120-134. In some embodiments, a cell can comprise an exogenous gene comprising a sequence that encodes for an enzyme with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or 100% sequence identity to any one of SEQ ID NO: 120-134.

[0104] In some embodiments, an enzyme described herein can comprise catalytic motif IV: a conserved proline. In some embodiments, an enzyme described herein can comprise a third domain having a sequence with a conserved proline. In some embodiments, an enzyme can comprise a sequence with at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or 100% sequence identity to any one of SEQ ID NO: 135-154. In some embodiments, a cell can comprise an exogenous gene comprising a sequence that encodes for an

enzyme with at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or 100% sequence identity to any one of SEQ ID NO: 135-154.

[0105] *Chlamydomonas reinhardtii*, a unicellular green algae, comprises two LPAAT genes: CrLPAAT1 and CrLPAAT2. The chlorophyte specific CrLPAAT2 enzyme localizes to ER membranes. Unlike canonical ER-located LPAATs, however, CrLPAAT2 prefers palmitoyl-CoA over oleoyl-CoA as the acyl donor substrate. In some embodiments, the amino acid sequence of CrLPAAT1 is SEQ ID NO: 12. In some embodiments, CrLPAAT1 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2. In some embodiments, the amino acid sequence of CrLPAAT2 is SEQ ID NO: 13. In some embodiments, CrLPAAT2 is expressed in cells by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10. Related species *Chlamydomonas schloesseri*, *Chlamydomonas incerta*, and *Chlamydomonas eustigma* have similar genes – *ChsLPAAT2*, *CiLPAAT2*, and *CeLPAAT2*, respectively. In some embodiments, the amino acid sequence of ChsLPAAT2 is SEQ ID NO: 14. In some embodiments, ChsLPAAT1 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 9. In some embodiments, the amino acid sequence of CiLPAAT2 is SEQ ID NO: 15. In some embodiments, CiLPAAT1 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10. In some embodiments, the amino acid sequence of CeLPAAT2 is SEQ ID NO: 53. In some embodiments, CeLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 38.

[0106] The marine microalgae genus *Nannochloropsis* has 4 LPAAT genes. Two of these genes, *NoLPAT3* and *NoLPAT4*, are critical for TAG biosynthesis. *NoLPAT3* and *NoLPAT4* are typically found around the perimeter of lipid droplets, although these two genes are unrelated to other lipid droplet-associated proteins. The location of *NoLPAT3* and *NoLPAT4* on the perimeter of lipid droplets enhances the accumulation of TAG, particularly during nutrient deprivation. In some embodiments, the amino acid sequence of *NoLPAT3* is SEQ ID NO: 16. In some embodiments, *NoLPAT3* is expressed in a cell by transformation with a plasmid comprising a sequence with at

least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 7. In some embodiments, the amino acid sequence of NoLPAT4 is SEQ ID NO 17. In some embodiments, is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

[0107] In the oilseed rape *Brassica napus*, one of the LPAAT genes involved in TAG biosynthesis is *BnBAT2*. *BnBAT2* is a prokaryotic-type LPAAT gene. In some embodiments, the amino acid sequence of BnBAT2 is SEQ ID NO: 18. In some embodiments, BnBAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3.

[0108] The cyanobacteria *Synechocystis sp.* LPAAT gene is referred to as *Sll1848*. Sll1848 incorporates C18:0 and C18:1 in the *sn-2* position, and is the major LPAAT specific to C16:0 in *Synechocystis sp.* In some embodiments, the amino acid sequence of Sll1848 is SEQ ID NO: 19. In some embodiments, Sll1848 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 5.

[0109] Species of green algae such as *Volvox carteri*, *Volvox africanus*, and *Volvox reticuliferus* also have similar LPAAT genes - *VcLPAAT2*, *VaLPAAT2*, and *VrLPAAT2*, respectively. In some embodiments, the amino acid sequence of VcLPAAT2 is SEQ ID NO: 20, or SEQ ID NO: 40. In some embodiments, VcLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 25. In some embodiments, the amino acid sequence of VaLPAAT2 is SEQ ID NO: 21. In some embodiments, VaLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11. In some embodiments, the amino acid sequence of VrLPAAT2 is SEQ ID NO: 55. In some embodiments, VrLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 57.

[0110] The LPAAT genes in *Escherichia coli* are referred to as *PlsC* genes. In *E. coli*, the *EcPlsC* mutant strain is characterized by a deficiency of LPAAT activity. The strain is also temperature-

sensitive, unable to grow at 42 °C. In some embodiments, the amino acid sequence of EcPlsC is SEQ ID NO: 22. In some embodiments, EcPlsC is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6.

[0111] The LPAATs of the microalgae *Prototheca moriformis* display a strong preference for the insertion of unsaturated fatty acids at the *sn*-2 position, rarely inserting saturates. In some embodiments, the amino acid sequence of PmLPAAT1 is SEQ ID NO: 23. In some embodiments, the amino acid sequence of PmLPAAT2 is SEQ ID NO: 24.

[0112] Species of green algae such as *Chlorella sorokiniana*, *Chlorella variabilis*, *Chlorella desiccata*, and *Chlorella vulgaris* also have similar LPAAT genes – *ChsoLPAAT2*, *ChvaLPAAT2*, *ChdeLPAAT2*, and *ChvuLPAAT2*, respectively. In some embodiments, the amino acid sequence of ChsoLPAAT2 is SEQ ID NO: 46. In some embodiments, ChsoLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31. In some embodiments, the amino acid sequence of ChvaLPAAT2 is SEQ ID NO: 47. In some embodiments, ChvaLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 32. In some embodiments, the amino acid sequence of ChdeLPAAT2 is SEQ ID NO: 49. In some embodiments, ChdeLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 34. In some embodiments, the amino acid sequence of ChvuLPAAT2 is SEQ ID NO: 56. In some embodiments, ChvuLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 58.

[0113] The multicellular green algae *Astrephomene gubernaculifera* comprises an LPAAT gene referred to as *AgLPAAT2*. In some embodiments, the amino acid sequence of AgLPAAT2 is SEQ ID NO: 41. In some embodiments, AgLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26.

[0114] The green algae *Edaphochlamys debaryana* comprises an LPAAT gene referred to as *EdLPAAT2*. In some embodiments, the amino acid sequence of EdLPAAT2 is SEQ ID NO: 42. In

some embodiments, EdLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27.

[0115] The unicellular green algae *Dunaliella salina* comprises an LPAAT gene referred to as *DsLPAAT2*. In some embodiments, the amino acid sequence of DsLPAAT2 is SEQ ID NO: 43. In some embodiments, DsLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28.

[0116] The colonial green algae *Scenedesmus sp.* comprises an LPAAT gene referred to as *SceLPAAT2*. In some embodiments, the amino acid sequence of SceLPAAT2 is SEQ ID NO: 44. In some embodiments, SceLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 29.

[0117] The colonial green algae *Volvulina compacta* comprises an LPAAT gene referred to as *VcomLPAAT2*. In some embodiments, the amino acid sequence of VcomLPAAT2 is SEQ ID NO: 83. In some embodiments, VcomLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 65.

[0118] The colonial green algae *Pleodorina japonica* comprises an LPAAT gene referred to as *PjapLPAAT2*. In some embodiments, the amino acid sequence of PjapLPAAT2 is SEQ ID NO: 86. In some embodiments, PjapLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 68.

[0119] The colonial green algae *Volvulina boldii* comprises an LPAAT gene referred to as *VbolLPAAT2*. In some embodiments, the amino acid sequence of VbolLPAAT2 is SEQ ID NO: 87. In some embodiments, VbolLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 69.

[0120] The colonial green algae *Gonium multicoccum* comprises an LPAAT gene referred to as *GmulLPAAT2*. In some embodiments, the amino acid sequence of GmulLPAAT2 is SEQ ID NO: 91. In some embodiments, GmulLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 73.

[0121] The colonial green algae *Gonium viridistellatum* comprises a LPAAT gene referred to as *GvirLPAAT2*. In some embodiments, the amino acid sequence of *GvirLPAAT2* is SEQ ID NO: 92. In some embodiments, *GvirLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 74.

[0122] The unicellular green algae *Micractinium conductrix* comprises a LPAAT gene referred to as *McLPAAT2*. In some embodiments, the amino acid sequence of *McLPAAT2* is SEQ ID NO: 45. In some embodiments, *McLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30.

[0123] The unicellular green algae *Dunaliella salina* comprises an LPAAT gene referred to as *DsalLPAAT2*. In some embodiments, the amino acid sequence of *DsalLPAAT2* is SEQ ID NO: 96. In some embodiments, *DsalLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 78.

[0124] The microalgae *Raphidocelis subcapita* comprises an LPAAT gene referred to as *RsLPAAT2*. In some embodiments, the amino acid sequence of *RsLPAAT2* is SEQ ID NO: 48. In some embodiments, *RsLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 33.

[0125] The heterotrophic green algae *Auxenochlorella protothecoides* comprises an LPAAT gene referred to as *ApLPAAT2*. In some embodiments, the amino acid sequence of *ApLPAAT2* is SEQ ID NO: 50. In some embodiments, *ApLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 35.

[0126] The green algae *Chloropicon primus* comprises a LPAAT gene referred to as *ChprLPAAT2*. In some embodiments, the amino acid sequence of *ChprLPAAT2* is SEQ ID NO: 51. In some embodiments, *ChprLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36.

[0127] The green algae class Pedinophyceae comprises an LPAAT gene referred to as *PedLPAAT2*. In some embodiments, the amino acid sequence of *PedLPAAT2* is SEQ ID NO: 54. In some embodiments, *PedLPAAT2* is expressed in a cell by transformation with a plasmid

comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 39. In some embodiments, PedLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 59. In some embodiments, PedLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60. In some embodiments, PedLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 61. In some embodiments, PedLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 62.

[0128] The green algae *Vitreochlamys sp. CL-2021* comprises an LPAAT gene referred to as *VitrLPAAT2*. In some embodiments, the amino acid sequence of *VitrLPAAT2* is SEQ ID NO: 84. In some embodiments, *VitrLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 66.

[0129] The green algae *Colemanosphaera charkowiensis* comprises an LPAAT gene referred to as *CchaLPAAT2*. In some embodiments, the amino acid sequence of *CchaLPAAT2* is SEQ ID NO: 85. In some embodiments, *CchaLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 67.

[0130] The green algae *Volvulina boldii* comprises an LPAAT gene referred to as *VbolLPAAT2*. In some embodiments, the amino acid sequence of *VbolLPAAT2* is SEQ ID NO: 87. In some embodiments, *VbolLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 69.

[0131] The green algae *Pandorina morum* comprises an LPAAT gene referred to as *PmorLPAAT2*. In some embodiments, the amino acid sequence of *PmorLPAAT2* is SEQ ID NO: 88. In some embodiments, *PmorLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 70.

[0132] The green algae *Volvox carteri* f. *weismannia* comprises an LPAAT gene referred to as *VcarfLPAAT2*. In some embodiments, the amino acid sequence of *VcarfLPAAT2* is SEQ ID NO: 89. In some embodiments, *VcarfLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 71.

[0133] The green algae *Eudorina cylindrica* comprises an LPAAT gene referred to as *EcyILPAAT2*. In some embodiments, the amino acid sequence of *EcyILPAAT2* is SEQ ID NO: 90. In some embodiments, *EcyILPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 72.

[0134] The green algae *Volvox ferrisii* comprises an LPAAT gene referred to as *VferLPAAT2*. In some embodiments, the amino acid sequence of *VferLPAAT2* is SEQ ID NO: 93. In some embodiments, *VferLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 75.

[0135] The green algae *Vitreochlamys aulata* comprises an LPAAT gene referred to as *VaulLPAAT2*. In some embodiments, the amino acid sequence of *VaulLPAAT2* is SEQ ID NO: 94. In some embodiments, *VaulLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 76.

[0136] The green algae *Chlamydomonas* sp. *CCAC2762_B* comprises a LPAAT gene referred to as *Ch_CCAC2762_LPAAT2*. In some embodiments, the amino acid sequence of *Ch_CCAC2762_LPAAT2* is SEQ ID NO: 95. In some embodiments, *Ch_CCAC2762_LPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 77.

[0137] The green algae *Microglena* sp. *YARC* comprises a LPAAT gene referred to as *MyarcLPAAT2*. In some embodiments, the amino acid sequence of *MyarcLPAAT2* is SEQ ID NO: 97. In some embodiments, *MyarcLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 79.

[0138] The green algae *Chlamydomonas* sp. *UWO_241* comprises a LPAAT gene referred to as *CuwoLPAAT2*. In some embodiments, the amino acid sequence of *CuwoLPAAT2* is SEQ ID NO:

98. In some embodiments, CuwoLPAAT2 is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 80.

[0139] The green algae *Chlamydomonas moewusii* comprises an LPAAT gene referred to as *CmoeLPAAT2*. In some embodiments, the amino acid sequence of *CmoeLPAAT2* is SEQ ID NO: 99. In some embodiments, *CmoeLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 81.

[0140] The green algae *Oophila amblystomatis* comprises an LPAAT gene referred to as *OambLPAAT2*. In some embodiments, the amino acid sequence of *OambLPAAT2* is SEQ ID NO: 100. In some embodiments, *OambLPAAT2* is expressed in a cell by transformation with a plasmid comprising a sequence with at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 82.

[0141] The green algae *Edaphochlamys debaryana_SRR13719274* comprises an LPAAT gene referred to as *EdLPAAT2*. In some embodiments, the amino acid sequence of *EdLPAAT2* (*Edaphochlamys debaryana_SRR13719274*) is SEQ ID NO: 101.

[0142] The colonial green algae *Volvulina steinii* comprises an LPAAT gene referred to as *VsteLPAAT2*. In some embodiments, the amino acid sequence of *VsteLPAAT2* is SEQ ID NO: 102.

[0143] The colonial green algae *Gonium octonarium* comprises an LPAAT gene referred to as *GoctLPAAT2*. In some embodiments, the amino acid sequence of *GoctLPAAT2* is SEQ ID NO: 103.

[0144] The unicellular green algae *Chlamydomonas nivalis* comprises an LPAAT gene referred to as *CnivLPAAT2*. In some embodiments, the amino acid sequence of *CnivLPAAT2* is SEQ ID NO: 104.

[0145] The green algae *Pycnococcus provasolii* comprises an LPAAT gene referred to as *PproLPAAT2*. In some embodiments, the amino acid sequence of *PproLPAAT2* is SEQ ID NO: 105.

[0146] The green algae *Bathycoccus prasinos* comprises an LPAAT gene referred to as *BpraLPAAT2*. In some embodiments, the amino acid sequence of *GoctLPAAT2* is SEQ ID NO: 106.

[0147] CrLPAAT1-ΔCST* is a *Chlamydomonas reinhardtii* LPAAT with a total of 30 amino acids (aa 12-41; CST signal) removed from CrLPAAT1. BnBAT2-ΔCST* is a *Brassica napus*

LPAAT with a total of 86 amino acids (aa 2-87) removed from BnBAT2. In some embodiments, the amino acid sequence of CrLPAAT1-ΔCST* is SEQ ID NO: 155. In some embodiments, the amino acid sequence of BnBAT2-ΔCST* is SEQ ID NO: 156.

TAG Oils of the Disclosure

[0148] Provided herein are TAG oil compositions having TAG species with a saturated fatty acid at the *sn*-2 position. In some embodiments, the TAG species is OPO.

[0149] The complexity and physical properties of an oil can be evaluated by the fatty acid profile of the TAG component of an oil and the TAG profile. The fatty acid profile is a measure of fatty acid composition and can be determined by subjecting an oil to transesterification to generate fatty acid methyl esters (FAMES) and subsequently quantitating fatty acid type by Gas Chromatography equipped with a Flame Ionization Detector (GC/FID). Accordingly, the fatty acid content of a TAG oil provided herein can be determined by GC/FID. Since TAGs comprise three fatty acids arrayed along the glycerol backbone in the triglyceride molecule, the number of possible distinct regioisomers of TAGs can be defined by the number of fatty acid species in the oil raised to the third power.

[0150] The genetic expression of an enzyme described herein can be modulated to enrich for OPO production. Expression of a gene described herein can be modulated singly or in combination with expression of one or more enzymes described herein, to enrich for OPO production. The genetic expression of an enzyme described herein can be modulated to enhance LPAAT activity. Expression of a gene described herein can be modulated singly or in combination with the expression of one or more enzymes described herein, to enhance LPAAT activity. In some embodiments, a cell described herein is modified to express an exogenous gene to enhance LPAAT activity. In some embodiments, a cell described herein is modified to delete an endogenous gene to enhance LPAAT activity.

[0151] Provided herein are methods of genetically modifying and cultivating a cell to enrich for production of an oil provided herein. Further provided herein are cells that are genetically modified to enrich for production of an oil described herein. A cell provided herein can contain recombinant nucleic acids operable to increase the expression of a gene described herein to enhance LPAAT activity. Further, a cell provided herein can exclude endogenous nucleic acids operable to decrease the expression of a gene described herein to enhance LPAAT activity. In some embodiments, a microalgal cell is modified to enrich for the production of an oil provided herein.

[0152] Oil compositions provided herein are produced by a microorganism, such as a microalgal cell. As such, an oil composition produced therefrom may comprise one or more sterol components that are characteristic of an algal oil. The amount of a sterol component in an oil can be determined on a weight-by-weight basis, e.g., mg of a sterol per 100 g of the oil. Sterol composition (i.e., a sterol profile) can be determined by mass spectrometry, for example, gas chromatography-mass spectrometry (GC-MS); liquid chromatography-mass spectrometry (LC-MS); tandem mass spectrometry (MS/MS), and coupled liquid and gas chromatography with subsequent flame ionization detection (LC-GC-FID). An example sterol profile as determined by GC/MS analysis of TMS esters of the non-saponifiable fraction of the RBD oil produced from CHK22 is shown in **Table 26**. Cholestan-3-ol(3a,5b) was used as an internal standard (ISTD).

Table 26: CHK22 RBD Oil Sterol Profile

Sterol Name	mg/100g	% w/w
Cholestan-3-ol (3a,5b) ISTD	** ISTD **	** ISTD **
Ergosta-7,22-dien-3-ol, (3 β ,22E)-, TMS derivative	9.288	0.009
Unknown 2	107.521	0.108
Ergosterol, TMS derivative	124.381	0.124
Unknown	69.057	0.069
Unknown	35.660	0.036
5.Xi.-Ergost-7-en-3 β -ol, TMS derivative	120.417	0.120
Unknown	10.601	0.011
9,19-Cyclolanostan-3-ol, 24-methylene-, (3B)-, O-TMS	5.366	0.005

[0153] In some embodiments, the oil contains a positive amount of ergosta-7,22-dien-3-ol, (3 β ,22E)-. In some embodiments, the amount of ergosta-7,22-dien-3-ol, (3 β ,22E)- in an oil provided herein is at least 2 mg, at least 5 mg, or at least 8 mg, or at least 9 mg per 100 g of the oil. In some embodiments, the amount of ergosta-7,22-dien-3-ol, (3 β ,22E)- in an oil is 1-100 mg, 2-30 mg, 3-20 mg, or 5-15 mg per 100 g of the oil.

[0154] In some embodiments, the oil contains a positive amount of ergosterol. In some embodiments, the amount of ergosterol in an oil provided herein is at least 50 mg, at least 100 mg, or at least 125 mg per 100 g of the oil. In some embodiments, the amount of ergosterol in an oil provided herein is at least 10 mg, at least 20 mg, at least 30 mg, at least 40 mg, at least 50 mg, at least 60 mg, at least 70 mg, at least 80 mg, at least 90 mg, at least 100 mg, at least 110 mg, at least 120 mg, at least 130 mg, at least 140 mg, at least 150 mg, at least 160 mg, at least 170 mg, at least 180 mg, at least 190 mg, or at least 200 mg per 100 g of the oil. In some embodiments, the amount of ergosterol in an oil is 10-50 mg, 50-100 mg, 100-150 mg, 150-200 mg, 10-100 mg, 100-200 mg, 100-2000 mg, or 10-200 mg per 100 g of the oil.

[0155] In some embodiments, the oil contains a positive amount of campesterol, a positive amount of β -sitosterol, or a positive amount of stigmasterol. In some embodiments, the amount of campesterol, β -sitosterol, or stigmasterol is no more than 5 mg per 100 g of the oil. In some embodiments, the oil does not contain campesterol. In some embodiments, the amount of campesterol in an oil provided herein is no more than 5 mg of campesterol per 100 g of the oil. In some embodiments, the oil does not contain stigmasterol. In some embodiments, the amount of stigmasterol in an oil is no more than 5 mg per 100 g of the oil. In some embodiments, the oil does not contain β -sitosterol. In some embodiments, the amount of β -sitosterol in an oil is no more than 5 mg per 100 g of the oil.

[0156] In some embodiments, the oil contains a positive amount of ergosta-5,8-dien-3-ol, (3 β)-. In some embodiments, the amount of ergosta-5,8-dien-3-ol, (3 β)- is at least 1 mg per 100 g of the oil. In some embodiments, the amount of ergosta-5,8-dien-3-ol, (3 β)- is at least 1 mg, at least 2 mg, at least 3 mg, at least 4 mg, at least 5 mg, at least 6 mg, at least 7 mg, at least 8 mg, at least 9 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least 35 mg, at least 40 mg, at least 45 mg, or at least 50 mg per 100 g of the oil. In some embodiments, the amount of ergosta-5,8-dien-3-ol, (3 β)- is 1-5 mg, 10-20 mg, 50-100 mg, 100-150 mg, 150-200 mg, 1-50 mg, 20-100 mg, 100-200 mg, or 20-200 mg per 100 g of the oil.

[0157] In some embodiments, the oil contains a positive amount of 5.xi.-ergost-7-en-3 β -ol. In some embodiments, the amount of 5.xi.-ergost-7-en-3 β -ol is at least 15 mg per 100 g of the oil. In some embodiments the amount of 5.xi.-ergost-7-en-3 β -ol is at least 20 mg per 100 g of the oil. In some embodiments, the amount of 5.xi.-ergost-7-en-3 β -ol is at least 1 mg, at least 2 mg, at least 3 mg, at least 4 mg, at least 5 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least 35 mg, at least 40 mg, at least 45 mg, at least 50 mg, at least 60 mg, at least 70 mg, at least 80 mg, at least 90 mg, at least 100 mg, at least 110 mg, at least 120 mg, at least 130 mg, at least 140 mg, at least 150 mg, at least 160 mg, at least 170 mg, at least 180 mg, at least 190 mg, or at least 200 mg per 100 g of the oil. In some embodiments, the amount of 5.xi.-ergost-7-en-3 β -ol is 1-50 mg, 20-50 mg, 50-100 mg, 100-150 mg, 150-200 mg, 20-100 mg, 100-2000 mg, or 20-200 mg per 100 g of the oil.

[0158] In some embodiments, the oil contains a positive amount of 9,19-cyclolanostan-3-ol,24-methylene-, (3 β)-. In some embodiments, the amount of 9,19-cyclolanostan-3-ol,24-methylene-, (3 β)- is at least 5 mg per 100 g of the oil. In some embodiments, the amount of 9,19-cyclolanostan-3-ol,24-methylene-, (3 β)- is at least 1 mg, at least 2 mg, at least 3 mg, at least 4 mg, at least 5 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least

35 mg, at least 40 mg, at least 45 mg, or at least 50 mg per 100 g of the oil. In some embodiments, the amount of 9,19-cyclolanostan-3-ol,24-methylene-,(3 β)- is 5-10 mg, 10-20 mg, 20-30 mg, 30-40 mg, 40-50 mg, 5-25 mg, 25-50 mg, or 5-50 mg of per 100 g of the oil.

[0159] TAG profile analysis of oil compositions provided herein can be conducted using liquid chromatography-mass spectrometry (LC-MS). TAG oils provided herein can be extracted from cells into a solution of 3:1 toluene/2-propanol (v/v) by means of mechanical disruption using ceramic beads and vigorous agitation. Filtered extracts can then be injected in an LC mass spectrometer for profiling.

[0160] Chromatographic separation of TAG regioisomers cannot be achieved by the LCMS method described. Instead, qualitative assessment of the regiospecificity of a predominant TAG species can be performed based on the abundance of diacylglycerol (DAG) ions resulting from fragmentation of the TAG species (**FIG. 1**). In LC-APCI-MS analyses of TAGs, the loss of the fatty acid at the *sn*-1 and *sn*-3 positions are energetically more favorable compared to the *sn*-2 position, which results in a greater abundance of the DAG ions formed from those fragments. In the case of an OOP regioisomer, an approximate 1:1 ratio of the OP DAG ion (*m/z* 577) and OO DAG ion (*m/z* 603) can be observed, as shown in **FIG. 1, Panel A**. In the case of the OPO regioisomer, a greater abundance of the OP DAG ion (*m/z* 577) can be observed, as shown in **FIG. 1, Panel B**.

[0161] **FIG. 1** demonstrates that TAG species of the same molecular weight show distinctive daughter ion ratios. The two TAG species, OOP and OPO in **Panel A** and **Panel B**, respectively, have identical mass/charge (*m/z*) ratios for the parent ion (859), but as illustrated, the molecules fragment very differently due to the effects of differing collision energies. Low energy collisions eliminate fatty acyl groups from the *sn*-1 or *sn*-3 position with greater frequency due to the relative inaccessibility of the acyl group at the *sn*-2 position. In the case of OOP, as shown in **Panel A**, low energy collisions result in daughter ions with *m/z* of 577 (OP) and 603 (OO), while less frequent high energy collisions result in the elimination of O from the *sn*-2 position also results in a daughter ion of *m/z* 577, OP. As a consequence, the resulting mass spectra of the daughter ions are the sum of all these fragments. The peak at 577 (OP) results from the more frequent elimination of O at *sn*-1 and the less frequent elimination of O at *sn*-2. Hence, the 577 fragment is roughly 1.5-2x the height of the 603 fragment (OO) resulting from the more frequent elimination of P at the *sn*-3 position. Alternatively, and as shown in **Panel B**, the OPO TAG species shows very different daughter ion ratios as the products of low energy collisions PO and OP have the same *m/z* of 577, while the daughter ion of the less frequently generated, high

energy collision representing OO has a m/z of 603. For this TAG species, the 577 peak height, resulting from the summation of the low energy derived PO and OP daughter ions, is substantially greater than that for the 603 fragment, which requires much higher energy to generate.

[0162] In some embodiments, the oil can have an OP:OO m/z ratio as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. In some embodiments, the oil can have an OP:OO m/z ratio of at least 1.6 as determined by the abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil. In some embodiments, the oil can have an OP:OO m/z ratio of at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2.0, at least 2.1, at least 2.2, at least 2.3 at least 2.4 at least 2.5, at least 2.6, at least 2.7, at least 2.8, at least 2.9, at least 3.0, at least 3.1, at least 3.2, at least 3.3, at least 3.4, at least 3.5, at least 3.6, at least 3.6, at least 3.7, at least 3.8, at least 3.9, at least 4.0, at least 4.1, at least 4.2, at least 4.3, at least 4.4, at least 4.5, at least 4.6, at least 4.7, at least 4.8, at least 4.9, or at least 5.0 as determined by abundance of DAG ions resulting from mass spectrometry fragmentation of the TAG component in the oil.

[0163] TAG regiospecificity can further be determined by a porcine pancreatic lipase assay. This method can be used to determine the identity of the *sn*-2 fatty acid of a TAG by incubating the TAG with porcine pancreatic lipase. The lipase reaction results in deacylation of the fatty acids at the *sn*-1 and *sn*-3 positions. The remaining *sn*-2-monoacylglycerols (MAGs) can then be isolated and identified by solid phase extraction followed by a direct transesterification method and analysis of fatty acid composition by GC/FID (gas chromatography/flame ionization detection).

[0164] Oil compositions provided herein can include a TAG component in which at least 40% of TAGs in the TAG component are TAG species having a saturated fatty acid at the *sn*-2 position. In some embodiments, at least about 50% of the TAGs in the TAG component is a TAG species having a saturated fatty acid at the *sn*-2 position. In some embodiments, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, or about 60% of the TAGs in the TAG component is a TAG species having a saturated fatty acid at the *sn*-2 position. In some embodiments, 40-45%, 45-50%, 50-55%, 55-60%, 40-50%, 50-60%, or 40-60% of the TAGs in the TAG component are TAG species having a saturated fatty acid at the *sn*-2 position. In some embodiments, the saturated fatty acid at the *sn*-2 position is palmitate.

[0165] Oil compositions provided herein can include a TAG component in which at least some of the acyl chains of the TAG component are C18:1. In some embodiments, at least about 50% of the acyl chains in the TAG component are C18:1. In some embodiments, at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, or at least about 70% of the acyl chains in the TAG component are C18:1. In some embodiments, at least about 50-55%, at least about 55-60%, at least about 60-65%, at least about 60-67%, at least about 65-70%, at least about 50-60%, at least about 50-67%, at least about 60-70%, or at least about 50-70% of the acyl chains in the TAG component are C18:1.

Microbial Oils

[0166] In some embodiments, an oil provided herein is obtained from a genetically modified microorganism, for example, microalgae, oleaginous yeast, or oleaginous bacteria. In some embodiments, the genetically modified microorganism is a genetically modified *Prototheca sp.* strain. In some embodiments, a genetically modified microorganism comprises an exogenous gene or exogenous nucleotides. Alternatively, or additionally, a genetically modified microorganism does not comprise an endogenous gene or endogenous nucleotides. Genetic modification methods described herein can be used to confer enriched oleic acid and/or OPO TAG production in a microorganism.

[0167] An oil provided herein can be obtained from microalgae, oleaginous yeast, or oleaginous bacteria. In some embodiments, the microorganism is a modified *Prototheca sp.* strain. A non-genetically modified *Prototheca sp.* strain can be produced by classical strain improvement strategies. A genetically modified *Prototheca sp.* strain can be produced by the over expression of endogenous/exogenous genes and/or deletion of an endogenous gene in the microorganism. In some embodiments, a microorganism provided herein is a genetically modified *Prototheca sp.* strain produced by classical strain improvement strategies to optimize for enriched oleic acid production (e.g., at least 50% oleic acid) and enriched OPO production (e.g., at least 70% OPO). In some embodiments, a microorganism provided herein is a genetically modified *Prototheca sp.* strain produced by classical strain improvement strategies to optimize for enriched palmitic acid production (e.g., at least 30% palmitic acid) and enriched OPO production (e.g., at least 70% OPO).

[0168] An oil provided herein can be produced from a *Prototheca* cell. The oil produced can be from a UTEX 1533 *Prototheca* strain cell. The oil produced can be from a cell of a strain that does not express an exogenous thioesterase.

[0169] In some embodiments, an oil provided herein is produced by microalgae. In some embodiments, the microalgae is a species of a genus selected from the group consisting of: *Chlorella* sp., *Pseudochlorella* sp., *Heterochlorella* sp., *Prototheca* sp., *Arthrospira* sp., *Euglena* sp., *Nannochloropsis* sp., *Phaeodactylum* sp., *Chlamydomonas* sp., *Scenedesmus* sp., *Ostreococcus* sp., *Selenastrum* sp., *Haematococcus* sp., *Nitzschia*, *Dunaliella*, *Navicula* sp., *Trebouxia* sp., *Pseudotrebouxia* sp., *Vavicula* sp., *Bracteococcus* sp., *Gomphonema* sp., *Watanabea* sp., *Botryococcus* sp., *Tetraselmis* sp., and *Isochrysis* sp. In some embodiments, the microalgae is *Prototheca* sp. In some embodiments, the microalgae are *P. moriformis*. In some embodiments, the microalgae is *P. wickerhamii*.

Genetic Engineering and Classical Strain Improvement

[0170] Production of TAGs oils having particular phenotypes can be achieved by genetic and non-genetic modification techniques of microorganisms. While genetic engineering techniques can be used to target phenotypes elicited in a host oleaginous microbe, classical strain improvement, and other non-genetic engineering techniques can be employed to further enhance these phenotypes. Similarly, classical strain improvement or other non-genetic engineering techniques can be employed to enhance certain phenotypes that have been selected by genetic engineering techniques. Phenotypes can include the elaboration of a particular fatty acid profile, a particular TAG profile, yield on carbon, growth productivity, volumetric oil accumulation (e.g., g oil/L culture), oil productivity (e.g., g oil/L culture day), and oil as a percent dry cell weight (DCW) as a measure of strain performance.

[0171] In some embodiments, a microalgal cell provided herein produces at least 50% lipid by dry cell weight. In some embodiments, a microalgal cell provided herein produces at least 60% lipid by dry cell weight.

[0172] For example, a microorganism that has been non-genetically modified to confer a first phenotype (e.g., enriched oleic acid production) can be genetically modified to confer a second phenotype (e.g., enriched OPO production). The resulting microorganism can then confer both the first and second phenotype (e.g., enriched oleic acid and OPO production).

[0173] Microorganisms provided herein can be produced by classical strain improvement strategies to select microorganisms having a desired phenotype, e.g., high oleic oil production. Classical strain improvement (also called “mutation breeding”) involves exposing organisms to

chemicals or radiation to generate mutants with desirable traits. Ultraviolet (UV) light can be used to introduce random mutations within a microorganism's nuclear genome. Chemical mutagens include compounds that inhibit or disrupt the biosynthetic processes of a microorganism, e.g., antibiotics, antifungals, or carcinogens. Non-limiting examples of chemical mutagens include ICR-191, ethyl methanesulfonate (EMS), and 4-nitroquinoline-1-oxide (4-NQO). Non-limiting examples of chemical mutagens also include acridine mutagens, amino acid analogs, fatty acid biosynthesis inhibitors, cholesterol biosynthetic inhibitors, mTOR inhibitors, and membrane solubilizing agents. Combinations of chemical mutagens can also be used simultaneously to induce mutagenesis. Following mutagenesis, selective or enrichment agents can be used to select or enrich strains of interest. Non-limiting examples of enrichment agents include L-canavanine, cerulenin, triparanol, clomiphene, clomiphene citrate, clotrimazole, terfenadine, fluphenazine, AZD8055, BASF 13-338, cafenstrole, clomiphene, PF-042110, and phenethyl alcohol.

[0174] Microorganisms provided herein having enhanced or altered lysophosphatidic acid acyltransferase activity can be produced by genetic engineering techniques, such as genetic recombination. Enhancement of lysophosphatidic acid acyltransferase activity can be achieved by enhancing the expression of an exogenous gene and/or reducing the expression of an endogenous gene in the microorganism. Enhancement of gene expression can be through overexpression of an endogenous gene in a microorganism or expression of an exogenous gene. Reduction of gene expression can be through deletion or removal of an endogenous gene in a microorganism.

[0175] For example, a non-naturally occurring microorganism provided herein can be genetically modified to enhance or alter lysophosphatidic acid acyltransferase activity in the microorganism. Enhancement or alteration of lysophosphatidic acid acyltransferase activity can be achieved by enhancing the expression of an exogenous LPAAT gene and/or reducing the expression of an endogenous LPAAT gene in the microorganism.

[0176] Methods provided herein include classical strain improvement and/or genetic engineering methods to improve strain productivity, carbon yield, oleic acid content, and OPO content. Glucose consumption rate can be a highly predictive indicator of lipid titer. As such, the glucose consumption rate can be used as an enrichment tool in the mutant selection process.

Polyol Applications and End Products

[0177] Oils described herein can be used as substrates in chemistries used to produce polyols, for example, hydroformylation/reduction or epoxidation and ring opening. Oleic acid moieties at the

sn-1 and sn-3 position, when subjected to the aforementioned chemistries, will result in diols, that, when formulated with one or more excipients for a variety of applications, including but not limited to, process oils (e.g., for tires), waxes, lubricants, other polyols, macrodiols, other polyesterdiols, can be used to create polyurethane products, e.g., hard foams, soft foams, cast polyurethanes, thermoplastic polyurethanes (TPUs), elastomers, adhesives, coatings, laminates, films, and dispersions. Polyurethane products can be used to construct aerospace, automotive, medical, electronic, building and construction goods; sporting goods or recreational equipment, e.g., skis, snowboards, sidewalls, boating equipment, kayaks; and other consumer goods, e.g., industrial containers, coolers, mattresses, leather goods, apparel, footwear, mannequins, and phone cases. These polyurethane applications can serve as sustainable alternatives to petroleum-based, non-renewable materials, such as acrylonitrile butadiene styrene (ABS), ultra-high molecular weight polyethylene (UHMWPE), or high density polyethylene (HDPE).

[0178] Oils provided herein can have improved production efficiency and a TAG composition that is enhanced for improved control of urethane chemistry. These characteristics of microbial oil can result in a greater degree of hydroxyl group (-OH) uniformity relative to oils with greater TAG heterogeneity (hence, lower purity) and/or diversity (e.g., oilseed or plant derived oils). Polyols derived from oils provided herein highly enriched in single, hydroxylated TAG species, can be preferable in generating polymers, including in instances where physical properties of a polymer can be compromised by molecular impurities, such as non-hydroxylated fatty acids or randomness in the regioselective insertion of fatty acid moieties on the glycerol backbone that may be present in oils having a more diverse or heterogeneous TAG profile.

[0179] Polyols described herein can be particularly useful for producing polyurethane materials. For example, oils provided herein can have relatively low TAG diversity, low fatty acid diversity, and the fatty acids present in the oils may be hydroxylated fatty acids. A higher ratio of hydroxylated fatty acids to non-hydroxylated fatty acids can allow for increased chemical reactivity. Oils having low TAG diversity and a high proportion of hydroxylated fatty acids can be especially desirable in the production of polyurethanes because hydroxylated fatty acids can participate in crosslinking reactions with isocyanates. Thus, polyols generated from highly hydroxylated fatty acids comprising the oil can yield polyurethane materials having superior properties.

Polyol Production

[0180] The hydroxyl group functionality of polyols can be introduced via a chemical conversion of a triglyceride oil. This conversion typically involves the presence of a double bond on an acyl

moiety of the fatty acid, which can then be converted to incorporate one or more hydroxyl groups using several different chemistries including, for example, epoxidation/ring opening, ozonolysis, and hydroformylation and reduction.

[0181] Epoxidation and subsequent ring opening across the carbon-carbon double bonds of an acyl chain can be carried out using a variety of reagents including, for example, water, hydrogen, methanol, ethanol, propanol, isopropanol, or polyols. Ring opening can be facilitated by a reaction with an alcohol, including, for example, β -substituted alcohols. Ring opening of epoxidized oil can also be effected through the use of a hydrogenation catalyst, such as nickel, to create a hydroxyl moiety.

[0182] Hydroformylation with synthesis gas (syngas) can be carried out using rhodium or cobalt catalysts to form the aldehyde at the olefinic group. The aldehyde can subsequently undergo reduction to an alcohol in the presence of hydrogen and a nickel catalyst to generate the polyol.

[0183] The hydroformylation chemistry results in the preservation of fatty acid length and the formation of primary hydroxyl group moieties. Primary hydroxyl group functionalities can be desirable in some PU applications due to increased reactivity compared to secondary hydroxyl group moieties. Hydroxyl groups introduced to olefinic groups in the acyl chain can participate in subsequent downstream chemistries, i.e., a reaction with an isocyanate moiety to form a urethane linkage or a reaction with methyl esters to form polyesters. Saturated fatty acids which do not contain double bonds cannot participate in crosslinking reactions with isocyanates. Hence, saturated fatty acids can compromise the structural integrity and degrade performance of the polymer produced therefrom.

[0184] In some embodiments, a polyol provided herein contains a substantial proportion of primary hydroxyl groups. In some embodiments, a polyol provided herein contains secondary hydroxyl groups. In some embodiments, a polyol provided herein can be modified to increase the proportion of primary hydroxyl groups.

[0185] Derivatives of TAG oils provided herein can be starting materials for producing polyols. Non-limiting examples of these TAG derivatives include fatty acids, fatty acid methyl esters, fatty acid ethyl esters, hydroxylated fatty acids, hydroxylated fatty methyl esters, and hydroxylated fatty ethyl esters. Non-limiting examples of polyols include polyester diols, polyether diols, hydrogenated polyols, hydroformylated polyols, and epoxidized, ring opened polyols. Alternatively, TAG oils provided herein, without further chemical modification, can be directly used as starting materials for generating polyols.

[0186] Fatty acid methyl esters (FAMES) can be generated through ester chemistry. For example, the TAG can be cleaved through transesterification into FAMES and glycerol. In turn, FAMES can be subjected to epoxidation and ring opening, for example, to create FAMES of alcohols. Alternatively, polyols can first be generated from a TAG through epoxidation and ring opening, for example, followed by transesterification, into FAMES of alcohols and glycerol. Glycerol and potassium methoxide catalysts can be removed by washing with water.

[0187] Catalysts, including potassium methoxide (KOCH_3), 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), Titanium(IV) isopropoxide (TIP), dibutyltin dilaurate (DBTDL), tris(pentafluorophenyl)borane (BCF), and potassium tert-butoxide, among others, can be utilized to re-esterify ester groups to alcohol moieties. The dual functionality of alcohol FAMES can be used to create polymer networks using only the methyl esters of the alcohol. Due to the polarity of the molecules (ester on one end and alcohol at the other end), the resulting polymer networks can elongate in a linear, unidirectional manner, and terminate in a single hydroxyl group.

[0188] Polymer networks can also be elongated in a bi-directional manner by incorporation of a diol, such as low molecular weight diols or the polyols provided herein. Non-limiting examples of diols include propylene glycol, alkyl diols, 1,4-butanediol, 1,3-propanediol, and 1,6-hexanediol. In some embodiments, diols can be produced using microbial hosts.

[0189] Hyperbranched polyols can be prepared to achieve a range of properties, such as molecular weight, viscosity, branching, and reactivity. For example, hyperbranched polyols can combine with isocyanates, ionogenic molecules, or hydrophobic compounds to produce higher order polyols.

[0190] In some embodiments, TAG oils described herein can be utilized to create materials for polymer applications. As shown in **FIG. 7**, TAG oils enriched in OPO can undergo chemical conversion to create oils containing functional groups, such as epoxy, ethoxy, methoxy, or hydroxy. Possible modifications of OPO include (from left to right): hydroformylation and reduction, resulting in a diol; epoxidation, resulting in the di-epoxy; and epoxidation followed by ring opening (e.g., with ethanol), resulting in a diol. TAGs enriched in OPO can allow for a variety of chemistries to be deployed around the carbon-carbon double bond of the oleic moieties, which can thereby produce numerous varieties of monomers for use as chain extenders in polymer chemistry applications.

[0191] In some embodiments, strains producing OPO enriched TAGs such as those described herein can be useful substrates for additional genetic modifications with a delta-12 fatty acid desaturase (FAH12). Upregulation of FAH12 expression in such an OPO accumulating strain can

increase ricinoleic acid production, thereby increasing levels of ricinoleic acid incorporated into TAGs containing oleic acid. As a consequence, such strains can thereby produce diols, similar to those described in **FIG. 7**, but in vivo without requiring additional chemical modifications. Non-limiting examples of oleate 12-hydroxylases include the *Lesquerella fendleri* FAH12, *Ricinus communis* FAH12, or *Claviceps purpurea* FAH12. An example of such a diol is shown in **FIG. 8**.

EXAMPLES

Example 1: Expressing *Chlamydomonas reinhardtii* (*CrLPAAT2*) in *P. moriformis* strain CHK22.

[0192] The microalgae *Prototheca moriformis* strain CHK22 (UTEX 1533) obtained from the University of Texas at Austin Culture Collection of Algae produces a fatty acid ratio of oleate:palmitate of roughly 2:1, which can be suitable for OPO production. However, the *P. moriformis* lysophosphatidic acid acyltransferases (LPAATs) exhibit a strong preference for the insertion of unsaturated fatty acids over saturated fatty acids at the *sn*-2 position. In this example, heterologous LPAAT genes were tested to identify genes that can efficiently direct palmitate (a saturated fatty acid) to the *sn*-2 position of TAGs produced by the microalgae.

[0193] The *C. reinhardtii* *CrLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK385 contained 5' and 3' homology arms to permit targeted integration of *CrLPAAT2* into the genome and is shown in **Table 1**.

Table 1: Integrative sequences for the transformation of *P. moriformis* with pCHK385 encoding *CrLPAAT2*.

SEQ ID NO:	Sequence
1	gctcttcgcttgccccgaccctcggtgatctgggagccctgcgcagcccttaaatcatctcagtcaggtttctatgttcaactgagcctaaaggg ctttcgtcacgcgcagcagcacacgtatatcggtacgcagtcctcagaagcggtagaacagttcgcaagccctcgctcggtcgaaacttgcg ccagtactattgaattaaatgaatgatcgatgagacgcgaactttgcagaatgccactgagtttggccagagaatgggagtgggcgccattc accatccgctgtgacggcctgattcgccgagacgatgaatggcgagaccagggagcggttgcgagccccgagccggtagcaggaataac ggctgcacaatcttctgtccaattactggcaaccattagaagagccggagcgcgttgaaattctgcaatcgagtaattttcgatcgggcgggc ctgctgaaccctaaggctcggcctatgtttaaggcgatccaagatgcacgcggccccaggcacgtgtctcaagcacaacccccagccttagttt cgagactttgggagatagcggcagatatctagtttggcattttgtatattaattaagctcgaatggagcgtctgatgcggtgcagcgtcggt gcagcactggcagtggtggtgggtgcacctatcgtcggcgcttggtcagctggctccgcctcctcgggcgcgccCTTCTTGCGC TATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAA CACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCT CCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCGATTGCAAAGACATTATAGCGAGCTAC CAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGAT CGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCAGTCACAACCCGCAAACCTGTACA TGctgtgcagggccttctgttctgctgcccgttcgcgcgaagatcagcgcctcatgacgaacgagacgtccgaccgccccctggtgcactt caccaccaacaagggctggtgatgaacgaccccaacggcctgtgtgtacgacgagaagagacgccaagtggcactgtacttcagtagacaacccgaa cgacaccgtctgggggacgccctgtgtgtgggcccacgcacgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaag cgcaacgactccggcgcttctccggctccatgtgtgtgactacaacaacacctccggtcttcaacgacaccatcgacccgcgccagcgtgc gtggccatctggacctacaacaccccgagtcgagggagcagtagcatctctacagcctggagcgcggtacaccttcaccgagtagcagaaga

SEQ ID NO:	Sequence
	gtcggatccaacgcatgttgatgcagtagcccggtgtgctgaaagtatggaaggatgggtgcattggctattcacatgcgctgccacccctttcg caggaaatgtgccggcatcgttgggtgcaccgatggggaaaatcgacgttcgaccactacatgaaaatgtatacctctgaagatgcagcaactg cgggtgcgaaacggataacgggttggtaggtgtatgtcgcagcatgtgctggattttcgggcaactccccctgccacggccattgcaggtgtc atgttgactggaggatacgaacttcggccgtcaaattcccagaggaggacccctctgggcccacattgtgccactgctcttcgcttgcgtttt cctgtgtgaaattgt

[0194] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:CrLPAAT2:CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, the bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the *DAO1b* locus via homologous recombination.

The *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by the boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics, while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics.

The *P. moriformis* PmAMT03 promoter, indicated by the boxed, italicized text, drives the expression of the *CrLPAAT2*. The initiator ATG and terminator TGA codons of the *CrLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated in bold italics.

The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0195] Strain Growth and Transformation

[0196] Unless indicated otherwise, strains described herein were grown in a 50-mL conical tube formats with shaking at 200 rpm at 28 °C for 96 hours for lipid production. Culture supernatants were harvested via centrifugation and washed once with MilliQ water. The resulting cellular pellet was lyophilized to dryness and subjected to direct transesterification to generate fatty acid methyl esters (FAMES) for subsequent quantitation and characterization by gas chromatography-flame ionization detection (GC/FID).

[0197] Strains utilized for transformation were grown in a vegetative growth medium for 24 hours prior to transformation. Cells were pelleted by centrifugation, resuspended in culture medium, and then re-centrifuged. The resulting cell pellet was re-suspended in culture medium and 5×10^7 cells plated to the appropriate selection medium and allowed to dry in a sterile biosafety cabinet. Transformants of CHK22 were generated via particle bombardment of previously plated cells using gold nanoparticles. Primary transformants were selected for ability to grow on plates containing sucrose as the sole carbon source. Transformants were grown in a

lipid production medium and their resulting biomass was processed as previously described for fatty acid analysis.

[0198] LCMS Analytical Method

[0199] TAG profile analysis was conducted using LCMS. TAGs were extracted from cells into a solution of 3:1 toluene/2-propanol (v/v), by means of mechanical disruption using ceramic beads and vigorous agitation. The filtered extracts were then injected into an Agilent 1290 Infinity II UHPLC system coupled to a 6470B triple quadrupole mass spectrometer and APCI ionization source.

[0200] LCMS Determination of Regioisomers

[0201] A qualitative assessment of the regiospecificity of a predominant TAG species of the resulting oil was performed based on the abundance of DAG ions resulting from fragmentation of the TAG species (**FIG. 1**).

Example 2: Determination of the *sn*-2 fatty acid profile following digestion with porcine lipase.

[0202] The fatty acid composition at the *sn*-2 position was determined after incubating the TAGs with porcine pancreatic lipase. The lipase reaction results in deacylation of the TAG at the *sn*-1 and *sn*-3 positions, thereby leaving *sn*-2-MAGs. The *sn*-2-MAGs were then isolated using Agilent Bond Elut NH2 propyl solid phase extraction (SPE) cartridges and subjected to fatty acid composition analysis by gas chromatography using a direct transesterification method.

[0203] Mass spectra and DAG ion ratios for the non-transgenic strain CHK22 and CHK22 transformed with pCHK385 (D552-1, D552-3, and D552-4) are shown in **FIG. 2** and **Table 2**.

FIG. 2 shows mass spectrum of OOP/OPO triglyceride. While regioisomers are not chromatographically separated by this analytical method, the higher ratio of *m/z* 577 (OP) to *m/z* 603 (OO) suggests a greater abundance of the OPO regioisomer compared to that of CHK22.

Table 2: Screen of primary transformants of CHK22 with pCHK385.

Sample	FAME Profiles									TAG Profile <i>m/z</i> 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK385-1	0.04	1.20	22.15	2.86	59.82	11.81	0.57	0.19	0.13	3.19
pCHK385-2	0.03	1.12	21.48	3.27	61.96	9.91	0.58	0.28	0.13	1.57
pCHK385-3	0.04	1.37	27.09	2.53	55.51	11.16	0.62	0.17	0.10	3.35
pCHK385-4	0.04	1.24	22.05	2.78	59.11	12.66	0.59	0.18	0.14	3.45

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

[0204] Table 2 shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK385. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization GC/FID. TAG analysis was conducted using the LCMS method as described above.

[0205] The fatty acid composition at the *sn*-2 position of the transgenic lines CHK22, D552; 1, 3, and 4 were further determined after incubating the TAGs with porcine pancreatic lipase. The results are shown in Table 3.

Table 3: Determination of the percentage of palmitate at *sn*-2 position following digestion with pancreatic porcine lipase, for the primary transformants of CHK22 with pCHK385.

		C16:0	C18:0	C18:1	C18:2
CHK22 expressing <i>CrLPAAT2</i>	Purified Tag Prep 1	22.69	3.07	59.63	11.02
	Purified Tag Prep 2	22.73	3.11	59.59	10.87
	sn2 Mag Eluate Prep 1	42.33	0.68	46.52	6.46
	sn2 Mag Eluate Prep 2	41.83	0.60	46.84	6.64
	Purified Tag Prep 1	28.42	2.40	54.63	10.63
	Purified Tag Prep 2	28.63	2.40	54.71	10.29
	sn2 Mag Eluate Prep 1	53.11	0.45	36.61	5.93
	sn2 Mag Eluate Prep 2	53.42	0.43	36.76	5.64
	Purified Tag Prep 1	22.52	25.67	59.02	12.19
	Purified Tag Prep 2	22.84	2.72	59.05	11.74
	sn2 Mag Eluate Prep 1	43.68	0.63	44.58	6.52
	sn2 Mag Eluate Prep 2	43.72	0.67	43.89	6.51
CHK22	Purified Tag Prep 1	27.68	3.43	60.22	5.24
	Purified Tag Prep 2	26.66	3.33	60.16	6.51
	sn2 Mag Eluate Prep 1	9.50	1.48	75.65	6.79
	sn2 Mag Eluate Prep 2	4.15	0.69	82.94	8.29

[0206] In **Table 3**, *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hours, 200-mL culture in 1-L flask at 200 rpm at 28 °C in lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. The fatty acid composition at the *sn*-2 position was determined with the porcine pancreatic lipase assay as described above. A corresponding bar graph showing the percentage of palmitic acid at the *sn*-2 position for various strains that had been transformed with pCHK385 is shown in **FIG. 5**. A graph showing LC-MS results for oil from CHK22 and strain D552-3 is shown in **FIG. 6**, showing that D552-3 has a similar molecular signature to CHK22, but that the amounts of palmitic acid in the *sn*-2 position had significantly increased.

Example 3: Expressing *C. reinhardtii* LPAAT1 (*CrLPAAT1*) in *P. moriformis* strain CHK22.

[0207] *C. reinhardtii* *CrLPAAT1* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK391 contained 5' and 3' homology arms to permit targeted integration of *CrLPAAT1* into the genome and is shown in **Table 4**.

Table 4: Integrative sequences for the transformation of *P. moriformis* with pCHK391 encoding *CrLPAAT1*.

SEQ ID NO:	Sequence
2	gctcttccgcttgcgcacccctcggtgatctgggagccctgcgcagcccttaaatcatctcagtcaggtttctatgttcaactgagcctaaagggttcttcacgcgcacgagcacacgtatatacggctacgcagctctcagaagcggtagaacagttcgcagccctcgctcggtcgaacacgtgcgcaggtactattgaattaaatgaatgatcgaatgagacgcgaacacttttcagaatgccactgagtttgccagagaatgggagtgccgcatcaccatccgctgtgcacggcctgattcgccgagacgatgaatggcgagaccaggagcggttcgagccccgagccggtagcaggaataacggtcgacaatcttctgtccaattactggcaaccattagaaagagccggagcggttgaattctgcaatcagtaattttctgatcgccggggcctgctgaacctaaaggctcggcctatgtttaaggcgatccaagatgcacgcggcccgaggcagctgtctcaagcacaacccagccttagtttcgagactttgggagatagcgcgagatctagtttggcattttgtatattaattaaagctcgcgaatggagcgctctgatcggtgcagcgtcggtcgagcactggcagtgctggctcgcctatcgctcgccctggctgactggctcccgccctcctcgccgcgcgcCTTTCTTGCGCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAACACCGTAGATGCTTCGACCCCGGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGGAGAGCGCTGTTTAAATAGCCAGGCCCGGATTGCAAAAGACATTATAGCGAGCTACCAAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTCTCGTTTCAGTCACAACCCGCAAACTGTACAATGctgctgcaggccttctgttctctgctgcccgccttcgccccaagatcagcgctccatgacgaacgagacgtccgaccgccctgtgacacttcaccccaacaagggttggtgatgaacgaccccaacggcctgtgtgacgacgagaaggacgccaagtggcactgtacttcagttacaacccgaacgacacgtctgggggacgccctgttctggggccacgccacgtccgacgactgaccaactgggaggacagcccatcgccatcgccccgaagcgcaacgactcggcgcttctcggctccatgtgtgtgactacaacaacacccctcggtcttcaacgacaccatcgaccgcgcagcgctgctggtgcatctggacctacaacccccggagtcggaggacgtacatctctacagcctggacggcggtacaccttcaccgagtaccagaagaaccccgctgtggccgcaactccacccagttccgcgacccgaaggctcttctgtgacgagccctccgaagtggtatcatgaccgggccaagtcccaaggactacaagatcgagatctactctcggacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggcttctcggctaccagtagtggtgccccggcctgacgaggtccccaccgagcaggacccagcaagtcctactgggtgatgttatctccatcaacccggcgcccgccggcgtccttcaaccagttacttctggcagcttcaacggcaccacttcgaggccttcgacaacagctcccgctgtgtgacttcggcaaggactactacgccctgcagaccttcttaacaccgaccgacctacgggagcgccctgggcatcgctgtggcctccaactgggagttactccgcttcgtgccaccaacccctggcgctcctcatgtccctcgtgcgaagttctccctcaaacaccgagtaccagggccaacccggagacggagctgatcaacctgaaggccgagccgactctgaacatcagaacgcggccctggagcggttcgccaacacaccaggttgacgaaggccaacagctacaacgtcgactgtccaacagcaccggcaccctggagttcagctggtgtacgccgtcaacacccagacgatctcaagtcctgtgtcaaggcctggaggaccccgaggagtaacctccgcatgggcttcgaggtgtccgctccttctcttgaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttccaac

[illegible]

[0208] The construct can be written as

5'DAO1b::CrTUB2::ScSUC2::PmPGH::CvNR::PmAMT03::CrLPAAT1::CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed, uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *CrLPAAT1*. The initiator ATG and terminator TGA codons of the *CrLPAAT1* are indicated in uppercase, bold italics, while the remainder of the gene is indicated in bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase, underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0209] Table 5 shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK391. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 5: Screen of primary transformants of CHK22 with pCHK391.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK391 -1	0.04	0.72	20.49	2.38	59.35	14.38	0.78	0.27	0.26	1.52
pCHK391 -2	0.04	0.71	20.25	2.30	59.33	14.75	0.73	0.26	0.26	1.52
pCHK391 -3	0.04	0.71	20.05	2.27	59.36	14.90	0.77	0.26	0.26	1.48
pCHK391 -4	0.03	1.08	24.60	2.93	59.71	9.39	0.55	0.29	0.15	1.59
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

Example 4: Expressing *Brassica napus* BnBAT2 in *P. moriformis* strain CHK22.

[0210] *B. napus* *BnBAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK384 contained 5' and 3' homology arms to permit targeted integration of *BnBAT2* into the genome and is shown in **Table 6**.

Table 6: Integrative sequences for the transformation of *P. moriformis* with pCHK384 encoding *BnBAT2*.

SEQ ID NO:	Sequence
3	gctcttccgcttccccacccctcggtgatctgggagccctgcgcagcccttaaatcatctcagtcaggtttctatgttcaactgagcctaaagggc tttcgtcacgcgcacgagcacacgtatctcggtacgcagtcctcagaagcggtagaacagttcgcaagccctcgctcggtcgaaaacttgcgcc agtactattgaattaaattaaatgatcgatgagacgcgaaacttttcagaatgccactgagtttcccagagaatgggagtgccgcaactcacc atccgctgtgcacggcctgattcgcgagacgatgaatggcgagaccaggagcggcttgcagcccccagccgtagcaggaataacggtc gacaatcttctgccaattactggcaaccattagaaagagccggagcgcgttgaattctgcaatcgagtaatttttcatgctggcgccgctgct gaacctaaagctccggcctatgtttaaggcgatcaagatgcacgcggcccccagccagcgtgtctcaagcacaacccagccttagtttcgaga ctttgggagatagcggcagatatctagttggcattttgtatattaattaagctcgaatggagcgtctgatgcgggtcagcgtcgggtcgagca cctggcagtgggcgctggggtcgccctatcgctcgccgctggctcagctggctcccgccctcctcgccgcgccgctCTTCTTTCGCTATGA CACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGCGCGCTGCATGCAACACCGA TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCCTCTTCGTTCAGTCACAACCCGCAAACCTGTACAAATGctgctcaggcct tctgttctgtgctgcccgttcgcccgaagatcagcgcctccatgacgaacgagacgtccgaccgccccctgggtgacattcaccaccaacaagg ctggatgaacgaccccaacggcctgtgtgtacgacgagaaggacccaagtggcactgtacttccagtacaacccgaacgacaccgtctggggga cgccctgttctggggccacgccacgtccgacgactgaccaactgggaggaacagcccatcgccatcgccccgaagcgcaacgactcggcgcc ttctccggctccatgtgtgtggactacaacaacacccctcggtcttcaacgacaccatcgaccgcgcagcgctgcgtggccatctggacctaca caccgcggagtcgagggagcagtagtactctctacagcctggagcggtctacaccttaccggtaccagaagaaccccgctgctggcgccaaact ccaccagttccgcgacccgaaggtcttctgtacgagccctccagaagtggatcatgaccgcggccaaagtcaccagactacaagatcgagatct actcttcgacgacctgaatgcctggaagctggagtcgcgttcgcaacgagggcttctcggctaccagtagtgcctggcctgctgaggt ccccaccgagcaggacccagcaagtcactactgggtgatgtcatctccatcaaccccgccgccccggcgccggtccttcaaccagtagtctg gcagcttaacgcgacccacttcgagcccttcgacaacagtcgccgctgggtgacttcgcaaggaactactacgccctgcagaccttctcaaac gaccgacctacgggagcgccctgggcatcgctgggctccaaactgggagtagtccgcttctgccccaccaacccctggcgctctccatgtccct cgtgcgaagttctcccaacacccagtagtaccagcccaacccggagagcgtgatcaactgaagcccgagccgactctgaacatcagcaacg ccggccccctggagccggttcgccaccaacaccagttgacgaagcccaacagctacaacgtcgaactgtccaacagcaccggcaccctggagttc gagctgtgtgtacgccgcaacaccaccagacgatctcaaggtcgtgttcgaggaccttccctgtgttcaaggccctggaggaccccgaggagt acctccgcatgggcttcgaggtgtccgctccttcttctgaccgcggaacagcaaggtgaagtgtggaaggagaacccctacttcaacca cgcgatgagcgtgaacaacacagccctcaagagcgagaacgacctgtctactacaagggtgtacgcttctgtagcagaacatcctggagctgtact taacgacggcgacgtcgtgtccaccaacacacttcatgaccacgggaacccctgggctcgtgcaacatgacgaggggtggaacacactgt tctacatcgacaagttccaggtgcgcgaggtcaagtgaactTAAGACGCCCCGCGCGCGCACCTGACCTGTTCTCTCG AGGGCGCCTGTTCTGCCTTGCGAACAAGCCCCCTGGAGCATGCGTGCATGATCGTCTCTGGCG CCCCGCCGCGCGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGCGCATTGAAATTGTT GCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGTTGAGATGGAGGTACAGGA GTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAAGGGTCGTTTCTGGCCAGCGAATG ACAAGAACAAGATTCCACATTTCCGTGTAGAGGCTTGCCATCGAATGTGAGCGGGCGGGCCG CGGACCCCGACAAAACCCCTTACGACGTGGTAAGAAAAACGTGGCGGGCAGTGTCCCTGTAGCC TGAAGACCAGCAGGAGACGATCGGAAGCATCACAGCACAAACCGGTgcagcagcagctcggatagtagca cacactctggagcgtggtcgtgtgatggactgttgcggccacacttgccttgacctgtgaatatacctgcgcgtttatcaaacagcctcagtggtttg atcttgtgtacgcgttttgcaggtgtgtagctgttgtgtctatttgcgaataccaccccgacatcccttccctgttcatatcgttgcaccccaaccg caacttatactagctgtcttctctacagcgtgtctctgtctctactgccccctgcacagccttgggttgggtccgctgtattctctgtgact gcaactgttaaacacagcactgcaatgtgatgcacgggaagtagtgggatgggaacacaaatggactaggaaataacaatcaGGCCGAC AGGACGCGCGTCAAAGGTGCTGGTCTGTATGCCCTGGCCCGCAGGTGCTTGTCTGCTGCTGTTA GTGATTCCGCAACCCCTGATTTTGGCGCTTATTTTGGCGGTGGCAAAACGCTGGCGCCCCGCGAGCCGG GCCGGCGCGGATGCGGTGCCCCACGGCTGCCGGAATCCAAGGGAGGCAAGAGCGCCCCGGGTCA GTTGAAGGGCTTTACGCGCAAGGTACAGCCGCTCCTGCAAGGCTGCGTGGTGAATGGACGTGC AGGTCTCTGCTGAAGTTCTCCACCGCTCACCAGCGGACAAAGCACCAGGTGTATCAGGTCCGTGTC ATCCACTCTAAAGAGCTCGACTACGACCTACTGATGGCCCTAGATTCTTCATCAAAAACGCCTGAGA

SEQ ID NO:	Sequence
	C A C T T G C C C A G G A T T G A A A C T C C C T G A A G G G A C C A C C A G G G G C C C T G A G T T G T C C T T C C C C C C G T G G C G A G C T G C C A G C C A G G C T G T A C C T G T G A T C G A G G C T G G C G G G A A A A T A G G C T T C G T G T G C T C A G G T C A T G G G A G G T G C A G G A C A G C T C A T G A A A C G C C A A C A A T C G C A C A A T T C A T G T C A A G C T A A T C A G C T A T T T C C T C T T C A C G A G C T G T A A T T G T C C C A A A A T T C T G G T C T A C C G G G G T G A T C C T T C G T G T A C G G G C C C T T C C C T C A A C C C T A G G T A T G C G C G C A T G C G G T C G C C G C G C A A C T C G C G C G A G G G C C G A G G G T T T G G G A C G G G C C G T C C C G A A A T G C A G T T G C A C C C G G A T G C G T G G C A C C T T T T T T G C G A T A A T T T A T G C A A T G G A C T G C T C T G C A A A A T T C T G G C T C T G T C G C C A A C C C T A G G A T C A G C G G C G T A G G A T T T C G T A A T C A T T C G T C C T G A T G G G G A G C T A C C G A C T A C C C T A A T A T C A G C C C G A C T G C C T G A C G C C A G C G T C C A C T T T T G T G C A C A C A T T C C A T T C G T G C C C A A G A C A T T T C A T T G T G G T G C G A A G C G T C C C C A G T T A C G T C A C C T G T T T C C C G A C C T C C T T A C T G T T C T G T C G A C A G A G C G G G C C C A C A G G C C G G T C G C A G C C A T G g a c g t g g c c t c g c c c g c g g g t g t c t c t c c a c c c c c t a c t a c t c c a a g c c a t c t g t c t c t c c a g t c t c c t g a t c c g a t c c c c a t c t c c a a g g g t g t g t c t t c g c c g t e t c t c c a a c c t g a t c a c t c c t c g a c g c g c t c c c g c g g g t g a c c c g c g e a c c t c c g g g t g c a g t g g t g t a c c g t c c a t c c g t t c g a c c c t t c a a g g t g a a c g a a g a a c t c c c g a c c g t g a c c g t g c g t c t c g a c t g t c c g g c g c c g c a c c c c g a g t c a c t a c c c g a c c c g a g a t c a a g t g t c t c t c c g c t g c g c g g a c t g t c t t g t c t g t g g c g g g c a t c t c g c c a t c g t g t a c t g t g t g t g a t c a t c g g c a c c c t g t g t g t g t c g a c c g t a c c g c g a a g t t c a c c a c t g c c a a g t g t c c a a g t g t c c t c a t c t c a t c a c c c t t c t a c a a g a c c g a c a t c c a g g g c t g g a g a a c c t g c c t c t c c g a c a c c c t g c g t g a c t g t c t c a a c c a c a g t c t t c t g g a c a t c t a c a c c t g t g t c c t g g g c a g t c t c a a g t t a t c t c a a g a c g g g c a t c t t c g t a t c c c g t a t c g g g t g g g c c a t g t c a t g a t g g g c g t g g t g c c c t g a a g c g a t g g a c c c c g t c c a g g t g g a c t g c t g a a g c g t g c a t g g a g c t g g t g a a g a a g g g c g c c t c g t g t t c t t c t c c g a g g g a c c c g t c c a a g g a c g g c g c c t g g g c c c t t c a a g a a g g g c g c c t t a c a t c g c c g c c a a g a c g g g c g t g c c g t g g t g c c a t a c c c t g a t g g g c a c g g g a a g a t a t g c c a c g g g t c c g a g g g c a t c t g a a c c a c g g c a c t g c g t g a t c a t c a c a a g c c a t c a c g g t c a a g g c c a c g t g t g t g c g a g g a g g c c g c a a c a a g a t c g c c a g t c a t g a a c t g t c t c T A G T a a t t a a g c a g a c a g a c a g c t g g a t a g t a t c g a c a c a c t c t g g a c c t g t c t g t g t g a t g a t g t t g c c g c c a c a c t t g c t g c c t t g a c t c t g a a t a t c c t g c c g t t t t a c a a c a c c t c a g t g t t t g a t e t t g t g t a c g c g t t t t g c g a g t t g t a g t g t t t g t a t t t g c g a a t a c c a c c c c a g a t c c c c t t c c t c g t t t a t a t c g t t g a t e c c a a c c g a a c t t a t c t a c g t g t c t g t a t c c c t a c g c g t g c t c t g t c t c t g t c a c t g c c c c t c g a c a g c c t t g t t t g g g c t c c g c t g a t t c t c t g t a c t g c a a c c t g t a a a c c a g c a c t g c a a t g c t a t g c a c g g g a a g t a g t g g a t g g g a a c a c a a a t g g a a g c g t c g t g t g g g a g c t g g a g t c g t g g g c t t g a c g a c g g c g t g c a g c t t t g c a g g a t g t g c t t g g c g t g c g c g t t a c g t c g t g g c t g a g a a a t a t g g c g a c g a a a c g t t g a c g g t g g g g c c g g c g g g c t g t g a t g c c a t a c g a t t g g g t a c g c g g c a t t g g a t g g g a t t g g t a g g c t t a t g g a g g g a t a a t a g t t t t g t c g g a t c c a a c g a t g t c g t t g t g c a c c a t g g g g a a a t c g a c g t t c g a c c a t a c a t g a a a t g t a t a c c t g c g a a g a t g a c c a a c t g c g g g t g c g a a a c g a t a a c g g t t g g t a g g t g t a t g t c g a c a t g t g t g g a t t t t g c g g g c a a c t c c c c t g c c a c g g c c c a t t g c a g g t g t a t g t t g a c t g g a g g a t a c g a a c t t t c g g c c g t c a a a t t c c a g a g g a g g a c c c c t t g g g c g a c a t t g t g c c c a c t g c t c t t c c g t g t g t t c t g t g a a a t t g t

[0211] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*BnBAT2*:CvNR::3'DAO1b. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by boxed italicized text, drives the expression of the *BnBAT2*. The initiator ATG and terminator TGA codons of the *BnBAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *C.*

vulgaris nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0212] Table 7 shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK384. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 7: Screen of primary transformants of CHK22 with pCHK384.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK384 -1	0.04	0.65	19.44	2.43	60.06	14.75	0.79	0.27	0.28	1.53
pCHK384 -2	0.03	0.97	23.19	2.91	60.48	10.10	0.58	0.28	0.18	1.57
pCHK384 -3	0.04	0.65	19.62	2.48	59.53	15.02	0.79	0.27	0.29	1.45
pCHK384 -4	0.04	0.65	19.35	2.40	59.82	15.07	0.79	0.27	0.29	1.52
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

Example 5: Expressing *Nannochloropsis* NoLPAT4 in *P. moriformis* strain CHK22.

[0213] *Nannochloropsis* NoLPAT4 was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK386 contained 5' and 3' homology arms to permit targeted integration of NoLPAT4 into the genome and is shown in Table 8.

Table 8: Integrative sequences for the transformation of *P. moriformis* with pCHK386 encoding NoLPAT4.

SEQ ID NO:	Sequence
4	gctcttccgcttgcgcacccctggtgatctgggagccctgcgcagcccttaaatcatctcagtcagggttctatgttcaactgagcctaaaggc tttgcgtcacgcgacgagcacagctatcggctacgcagctctctcagaagcggtagaacagttcgaagccctcgctcggtcgaaaacttgcgcc agtactattgaattaaattaatgatcgaaatgagacgcgaacttttgcagaatgccactgagtttgcagagaaatgggagtgccgcatcacc atccgctgtgcacggcctgattccgagacgatgaatggcgagaccaggagcggcttgcgagcccgagccggtagcaggaataacggtc gacaatcttctgtccaattactggcaaccattagaaagagcggagcgttgaaattctgcaatcgagtaattttcgatgcggcggtgctgct gaacctaaagctcggcctatgtttaaggcgatccaagatgcacgcggcccgagcagtggtctcaagcacaaccccgagccttagtttcgaga ctttgggagatagcggcagatatctagtttggcattttgtatattaattaagtctcgcaatggagcgtctgatgcggtgcagcgtcggctgcagca cttggcagtggtgcgtgggtgcgcctatcgtcgcggcctggtcagctggctccgcctcctgcggcgccgCTTTCTTGCGCTATGA CACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAACACCGA

[illegible]

SEQ ID NO:	Sequence
	<i>ccctccgagctgcccctgctgaaggccgctgccccacgaggtgcacatcaacatgaagcctgggacatcaagaccacccccctgctgcgcgag</i> <i>aaccccccgaccgcgcgagcagttcctgcgcgagtccttcgaccgcaaggaggccctgctgaccgcttctactccaacaacaccgacacctccg</i> <i>gcggcggcctgaagcagacctcctcctgctggtgctcctcctcctcccccttccaactcctccgccccctgggttccctccttctcctccccctc</i> <i>gacctcctggtcctcctacaacgagcccggtgtacgtgcacaaccccactggacctacggcaaggccctgctgggcaacgggcatcgccatggc</i> <i>cctcgtgctgatgctggcctgctgctgcccgcctggccttctgcggctactccatcggtggttctgctggtgacgtgaacctcctggtgggaggg</i> <i>cttcgacacccctggagctgaacctggcctggtggtggggcgccaggcgggccagaccaagcaggagTAG</i><u>ttaattaagcagcagcagctcggg</u> <u>tagtatcgacacactctggacgctgctgctggtgactgttgcgccacactgtgctgctgacctgtgaatatccctgcgcgtttatcaaacagcctcagt</u> <u>gtgtttgatcttgtgtgtacgcgcttttgcgagttgctagctgctgtgctgctatttgcgaataccacccccagcatccccctccctcgtttcatatcgcttgcatccca</u> <u>accgcaacttatctacgctgtcctgctatccctcagcgctgctcctgctcctgctcactgccccctcgacagccttggtttgggtccgctgtattctcctggt</u> <u>actgcaacctgtaaacagcactgcaatgctgatgcacgggaagttagtggtggatgggaacacaaatggaagcgtctgctgttgggagctggagctgtg</u> <u>ggcttgacgacggcgtgcagctcttgaggatgtgctggcgtgctgcttcacgtcgtggctgagaaatattggcgacgaaaccttgacggcgtgg</u> <u>ggccggcggtgtggtggtgcatcagcattgggtacgcggcattggatgggattggttagcttatggaggataatagagttttgtcggtatcca</u> <u>acgcatgttgatcagtagcccggtgtgctgaaagttaggaaggatggtgcattggctattcacatgcgctgccaccccccttttcgaggaaatgt</u> <u>gccggcatcgttgggtgcacgatggggaatacgacgttcgaccactacatgaaatgtatacctctgaagatgcagcaactcggggtgcgaaa</u> <u>cggataacgggtttggtaggtgtatgtcgacgatgtgctggattttgcgggcaactccccctgccacggccattgcaggtgtcatgttgactggag</u> <u>gatacgaaatttcggcgtcaaatccagaggaggacccccctctgggcccagattgtgccactgctcttcgctgtgttctcgtgtgaaattg</u> <u>t</u>

[0214] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*NoLPAT4*:CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *NoLPAT4*. The initiator ATG and terminator TGA codons of the *NoLPAT4* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0215] **Table 9** shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK386. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 9: Screen of primary transformants of CHK22 with pCHK386.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK386-1	0.03	1.18	21.97	3.22	61.19	10.16	0.60	0.28	0.13	1.50
pCHK386-2	0.04	1.18	20.89	3.36	61.46	10.84	0.57	0.27	0.13	1.46
pCHK386-3	0.04	1.15	20.68	3.57	61.62	10.74	0.59	0.28	0.13	1.55
pCHK386-4	0.04	1.18	20.80	3.48	61.42	10.86	0.58	0.27	0.13	1.53
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

Example 6: Expressing *Synechocystis* sp *Sll1848* in *P. moriformis* strain CHK22.

[0216] *Synechocystis* sp *Sll1848* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK387 contained 5' and 3' homology arms to permit targeted integration of *Sll1848* into the genome and is shown in **Table 10**.

Table 10: Integrative sequences for the transformation of *P. moriformis* with pCHK387 encoding *Synechocystis* sp *Sll1848*.

SEQ ID NO:	Sequence
5	<code> gctcttccgcttgcggcaccctcggtgatctgggagccctgcgcagcccttaaatcatctcagtcagggtttctatgttcaactgagcctaaagggtc ttctgtcacgcgcagcagcacggtatctcggctacgcagctctctcagaagcggtagaacagttcgcaagccctcggtcggtcgaaaacttgcgc agtactattgaattaaatgaatgatcgaatgagacgcgaaacttttgcagaatgccactgagtttcccagagaatgggagtgccgcaattcacc atccgctgtgcacggcctgattcgcgcgagacgatgaatggcgagaccaggaggcggcttgcgagccccgagccggtagcaggaataacggtc gacaatcttctgtccaattactggcaaccattagaaagagccggagcgggttgaattctgcaatcgagtaatttttcgatgcggcgggcctgct gaacctaaagctccggcctatgttttaaggcgatccaagatgcacgcggcccgagcagctgtctcaagcacaacccagccttagtttcgaga ctttgggagatagcggcagatatctagtgttgcattttgcataaataaagctcgaatggagcgcctgtgatgcgggtcagcgtcggtgcagca cctggcagtgggcgctggggtcgccctatcgctcggcgccctggtcagctggctcccgccctctgcggcgccgcCTTTCCTTGCCTATGA CACTTCCAGCAAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAACACCGA TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGGCGCCTCTTCCTCTTCGTTTCAGTCACAACCCGCAAACCTGTACATAATGctgctcaggcct tctgttctgtgctggcggcttgcggccaagatcagcgcctccatgacgaacgagacgtccgaccgccccctgggtgcacttcaccccaacaagggt ctggatgaacgaccccaacggcctgtggtacgacgagaaggacgcaagtggcactgtacttccagtacaacccgaacgacaccgtctggggga cgccctgttctggggccacgccacgtccgacgacctgaacaaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcc ttctcggctccatgtgtgtggaactacaacaacacctccggcttcttcaacgacaccatcgacccgcgccagcgctcggtggccatcttgacctacaa caccgggagtcggaggagcagtagctctctacagcctggacggcggtctacaccttcaccgagtaccagaagaaccccgctggtggcgccaact ccacccagttccgcgacccgaaggtcttctgtacgagccctcccaagtggtatcatgaccgcggccaagtcaccaggactacaagatcgagatct actcctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggtctcctcggtaccagtagagtgccccggcctgacgaggt cccaccgagcaggaccccaagcagtcactgtgggtgatgttcctccatcaacccggcgccccggcgcggtccttcaaccagctacttgctg gcagcttaacggcaccacattcgaggccttcgacaacagctcccgctggtggtgacttcggcaaggactactacgccctgcagaccttcttaacacc gaccgacctacgggagcgccctgggcatcgctggggcctccaactgggagtaactcgccttcgtgcccaacacccctggcgctcctccatgtccct cgtgcgaagtctcctcaacaccgagtagcagggccaacccggagagcgtgatcaacctgaaggcgagccgagctctgaacatcagcaacg ccggccctggagccggttcgcccaacaccacgtgacgaaggccaacagctacaacgtcagctgtccaacagcaccggcaccctggaggtc </code>

in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *Sll1848*. The initiator ATG and terminator TGA codons of the *Sll1848* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0218] Table 11 shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK387. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 11: Screen of primary transformants of CHK22 with pCHK387.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK387 -1	0.03	0.81	19.20	2.44	60.34	14.56	0.76	0.27	0.23	1.60
pCHK387 -2	0.00	0.24	16.85	5.74	63.56	4.63	2.04	0.07	0.18	1.60
pCHK387 -3	0.04	0.73	17.67	2.02	58.43	18.37	0.84	0.22	0.25	1.50
pCHK387 -4	0.04	0.83	19.40	2.43	60.13	14.54	0.74	0.27	0.23	1.55
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

Example 7: Expressing *Escherichia coli* EcPlsC in *P. moriformis* strain CHK22.

[0219] The *E. coli* *EcPlsC* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK388 contained 5' and 3' homology arms to permit targeted integration of *EcPlsC* into the genome and is shown in **Table 12**.

Table 12: Integrative sequences for the transformation of *P. moriformis* with pCHK388 encoding *Escherichia coli* *EcPlsC*.

SEQ ID NO:	Sequence
6	gctcttccgcttccccacacctcgatctgggagccctgcgcagcccttaaatcatctcagtcagggttctatgttcaactgagcctaaagggc tttcgtcacgcgcagcagcacacgtatctcggtacgcagtcctcagaagcggtagaacagttcgcaagccctcgctcggtcgaaaacttgcgcc agtactattgaattaaattaaatgatcgatgagacgcgaaacttttgcagaatgccactgagtttcccagagaatgggagtgccgacctacc atccgctgtgcacggcctgattcgcgagacgatgaatggcgagaccaggagcggcttgcagccccgagccgtagcaggaataacggtc gacaatcttctgccaattactggcaaccattagaaagagccggagcgcgttgaattctgcaatcgagtaatttttcatgctggcgggcctgct gaacctaaagctccggcctatgtttaaggcgatccaagatgcacgcggcccgagccagctgtctcaagcacaacccagccttagtttcgaga ctttgggagatagcggcagatatctagttggcattttgtatattaattaagtctcgcaatggagcgtctgagtcgggtcagcgtcggtcgagca cttggcagtgggcgtgggtcgccctatcgctcgccgctggctcagctggctcccgctcctcgccgcgccCTTCTTTCGCGTATGA CACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGCGCGTGCATGCAACACCGA TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCCTCTTCGTTCAGTCACAACCCGCAAACCTGTACAAATGctgctcaggcct tctgttctgtgctggcggcttcgccccaagatcagcgtccatgacgaacgagacgtccgaccgccccctgggtgacattcccccaacaaggg ctggatgaacgaccccaacggcctgtgtgtacgacgagaaggacccaagtggcactgtacttccagtacaacccgaacgacacgtctggggga cgccctgttctggggccacgccacgtccgacgactgaccaactgggaggaacagcccatcgccatcgccccgaagcgcaacgactcggcgcc ttctccggctccatgtgtgtggactacaacaacacctccggcttctaacgacaccatcgaccgcgcagcgctgcgtggccatctggacctaca caccccgagtcgagggagcagtagtactctctacagcctggagcggtctacaccttaccgagtagcaagaaccccgctgctggcgccaact ccaccagttccgcgacccgaaggtcttctgtacgagcctccagaagtggatcatgaccgcggccaagtcaccagactacaagatcgagatct actcttcgacgacctgaatcctggaagctggagtcgcgttcgccaacgagggcttctcggctaccagtagtgcccgccctgctgaggt ccccaccgagcaggacccagcaagtcactggtgtgtatctctccatcaaccccgccgccccggcgccggtccttcaaccagtagtctgctg gcagcttaacgcgacccacttcgagccttcgacaacagtcgccgctgtgtgacttcggaaggaactactacgccctgcagaccttctcaaac gacccgacctacgggagcgccctgggcatcgctgggctcccaactgggagtagtccgcttcgtgcccaacacccctggcgctctccatgtccct cgtgcgaagtctctcccaacacccagtagtaccagccaacccggagtagctgatcaactgaagcccgagccgactctgaacatcagcaacg ccggccccctggagccggttcgccaccaacaccagttgacgaagccaacagctacaacgtcgaactgtccaacagcaccggcaccctggagttc gagctgtgtgtacgccgtaacaccaccagacgatctcaagtcctgtgttcgaggacctctccctgtgttcaaggccctggaggaaccccgagggt acctccgcatgggcttcgaggtgtccgctctcttcttctgaccgcggaacagcaaggtgaagtgtggaaggagaacccctacttcaacca cgeatgagcgtgaacaacacagccctcaagagcgagaacgacctgtctactacaagggtgtacgcttctgtgaccagaacatcctggagctgtact taacgacggcgacgtcgtgtccaccaacacctacttcatgaccagcggaacgccctgggctcgtgaacatgacgacggggtggaacacctgt tctacatcgacaagttccaggtgcgcgaggtcaagtgaactTAAGACGCCCCGCGCGCGCACCTGACCTGTTCTCTCG AGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGCATGATCGTCTCTGGCG CCCCGCCGCGCGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGCGCATTGAAATTGTT GCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGTTGAGATGGAGGTACAGGA GTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAAGGGTCGTTTCTGGCCAGCGAATG ACAAGAACAAGATTCCACATTTCCGTGTAGAGGCTTGCCATCGAATGTGAGCGGGCGGGCCG CGGACCCCGACAAAACCCCTTACGACGTGGTAAGAAAAACGTGGCGGGCAGTGTCCCTGTAGCC TGAAGACCAGCAGGAGACGATCGGAAGCATCACAGCACAAACCGGTgcagcagcagctcggatagtagca cacactctggagcgtggtcgtgtgatggactgttgcggccacacttgccttgacctgtgaatatacctgcgcgtttatcaaacagcctcagtggtttg atcttgtgtacgcgttttgcaggtgtgtagctgtgtgtctatgttgcgaataccaccccgacatcccttccctgcttcatatcgcttgatcccaaccg caacttatactacgtctcttctctacgcgtctctctgctctctactgccccctcgacagccttggttgggtccgctgtattctctggtact gcaacctgtaaacagcactgcaatgctgatgcaggggaagtagtgggatgggaacacaaatggactaggaaataacaatcaGGCCGAC AGGACGCGCGTCAAAGGTGCTGGTCTGTATGCCCTGGCCCGGACGTCGTTGCTGCTGCTGTTA GTGATTCCGCAACCCCTGATTTTGGCGCTTATTTTGGCGTGGCAAAACGCTGGCGCCCGCGAGCCGG GCCGGCGCGGATGCGGTGCCCCACGGCTGCCGGAATCCAAGGGAGGCAAGAGCGCCCGGGTCA GTTGAAGGGCTTTACGCGCAAGGTACAGCCGCTCCTGCAAGGCTGCGTGGTGAATGGACGTGC AGGTCTCTGCTGAAGTTCCTCCACCGCTCACCAGCGGACAAAGCACCAGGTGTATCAGGTCCGTGTC ATCCACTCTAAAGAGCTCGACTACGACCTACTGATGGCCCTAGATTCTTCATCAAAAACGCCTGAGA

SEQ ID NO:	Sequence
	<i>CACTTGCCCAGGATTGAAACTCCCTGAAGGGACCACCAGGGGCCCTGAGTTGTTCTTCCCCCGTGGCGAGCTGCCAGCCAGGCTGTACCTGTGATCGAGGCTGGCGGGAAAATAGGCTTCGTGTGCTCAGGTCATGGGAGGTGCAGGACAGCTCATGAAACGCCAACAAATCGCACAAATTCATGTCAAGCTAATCAGCTATTTCTCTTCACGAGCTGTAATTGTCCAAAAATTCTGGTCTACCGGGGGTGATCCTTCGTGTACGGGCCCTTCCCTCAACCTAGGTATGCGCGCATGCGGTGCGCCGCGCACTCGCGCGAGGGCCGAGGGTTTGGGACGGGCGGTCCCGAAATGCAGTTGCACCCGGATGCGTGGCACCTTTTTTGGCGATAATTTATGCAATGGACTGCTCTGCAAAATTCTGGTCTGTGCGCAACCCTAGGATCAGCGGCGTAGGATTTCGTAATCATTTCGTCTGATGGGGAGCTACCGACTACCCTAATATCAGCCCGACTGCCTGACGCCAGCGTCCACTTTTGTGCACACATTCCATTTCGTGCCCAAGACATTTCAITGTGGTGCGAAGCGTCCCCAGTTACGCTACCTGTTTCCCGACCTCCTTACTGTTCTGTCGACAGAGCGGGCCACAGGCCGGTGCAGCC</i> <i>ATGctgtacatctccgctgatcaccgtgatctactccatctggtgtgctgttgcctcatctactgctgttcccccgaacc</i> <i>caaagcagtcgccaccttggccacatgttgcgcgctgccccctgttgcgctgaagggtgagtgccgaagcccaaccgacgcgagtccta</i> <i>ggccaacgccatctacatgcccaaccacagaacaactacgacatgggtgacgcctccaacatcgtgcagccccccacctgacctgggcaaga</i> <i>agtcctgtgtgattcccttcttggccagctctactggtgacgggcaacctgctgatcgaccgcaacaaccgcaacaggcccaaggccaccatc</i> <i>gccgaggtggtcaaccactcaagaagcgccgcatctccatctggatgttccccgagggcaccgcctcccgccggcggtgctgccccttcaaga</i> <i>cggcgccctccacgcgcctatcgccggcggtgccatcatccccgtgtgctgtgcccaccctcccaacaagatcaactgaaccgcctgcacaa</i> <i>cggcctggtgatcgtggatgctgccccatcgacgtgtccagtagggcaaggaccaggtgctgcgagtgccgccactgccctccatcatg</i> <i>gagcagaagatcgccgagctggacaaggaggtggcgagcgcgaggccggcggaagggtg</i><i>TAG</i><i>tttaattaagcagcagcagctcgatagtat</i> <i>cgacacactctggacgctgctgctgtgatgactgttgcgccacactgctgacctgtgaatatccctgcgccttttatcaaacagcctcagtgctttt</i> <i>gatcttgtgtgtacgcgcttttgcgagttgctagctgcttgcctatttgcgaataccacccccagcatccccctccctcgtttcatatccttgcattcccaaccg</i> <i>caacttatctacgctgctctgctatccctcagcgtgctctgctctgctcactgccccctgcacagccttggtttggctccgctgtattctcctgtaactgc</i> <i>aacctgtaaacaccagcactgcaatgctgatgcacgggaagtgtggatgggaacacaaatggaagcgtctgctgttgggagctggagtcgtgggctt</i> <i>gacgacggcgctgcagctcttgcaggatgtgctggcggtgcggttcacgtcgtggctgagaaatattggcgacgaaacgttgacggctggggcc</i> <i>ggcggtgctggatgccatagcattgggtacggccattggatgggattggtaggcttatggaggataatagagtttttgcggatcccaaccg</i> <i>atgttgatgcagtagcccggtgtgctgaaagtatggaaggatgggtcattggttaacatgcgctgccaccccccttttcgaggaaatgtgcgg</i> <i>gcctcgttgggtgacccgatggggaatcgacgttcgaccactacatgaaatgtatacctctgaagatgcagcaactcggggtgcgaacggga</i> <i>taacggttggtaggtgtatgtcgacatgtgctggtatttgcgggcaactccccctgccacggccattgcaggtgtcatgttgaactggaggata</i> <i>cgaactttggcgctcaaatccagaggaggacccccctgggccgacattgtgccactgctcttcgctgtgtttcctgtgtgaaattgt</i>

[0220] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:EcPlsC:CvNR::3'DAO1b. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *EcPlsC*. The initiator ATG and terminator TGA codons of the *EcPlsC* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0221] **Table 13** shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK388. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 13: Screen of primary transformants of CHK22 with pCHK388.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12: 0	C14: 0	C16: 0	C18: 0	C18: 1	C18: 2	C18:3 alpha	C20: 0	C20: 1	
pCHK388 -1	0.03	1.19	24.48	3.30	60.71	8.20	0.53	0.27	0.11	1.55
pCHK388 -2	0.04	1.23	21.52	2.79	59.05	13.08	0.64	0.22	0.13	1.52
pCHK388 -3	0.04	1.20	21.59	2.81	59.43	12.62	0.62	0.22	0.13	1.54
pCHK388 -4	0.03	1.18	23.32	3.24	60.90	9.14	0.55	0.28	0.12	1.59
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

Example 8: Expressing *Nannochloropsis* NoLPAT3 in *P. moriformis* strain CHK22.

[0222] The *Nannochloropsis* NoLPAT3 was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK453 contained 5' and 3' homology arms to permit targeted integration of NoLPAT3 into the genome and is shown in **Table 14**.

Table 14: Integrative sequences for the transformation of *P. moriformis* with pCHK453 encoding *Nannochloropsis* NoLPAT3.

SEQ ID NO:	Sequence
7	cctcaactgcagcctgggaacctctccgggcaggcgatgtgcgtgggttgccctcttgccacggctctacaccgtcgagtacgcatgagge ggtgatggctgtgcgttgccactctgcccagagacggcaagtcgtccatctctgctgtgtggcgagcgtgcagcagtcctctgcagcag atgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttcttaaagcaaatgactgctgattgaccagatactgaacgtgatt tcgtccagatgcacagatagcgaccatgttgcgtgctgaaatctggattccgaattcgacctggcgctccatccatgcaacagatggcga cactgtttacaattctgtcaccatcgccatggagcaggtccacttagattcccgatcacccacgcacatctcgctaatagtcattcgtctgtctt cgatcaatcgaagtgtgtgcatggatcttggtgacgatgcggtatgggttgcgcgctggctgcagggctgcgaaggcaagctaacca gctcctctcccgacaatactctgcaggcaagccggtcacttgccttcagattgccaataaactcaattatggcctctgtcatgccatcatgg gtctgatgaatggtaacgtcgtgtctgacgttccccagcctctggcgctccctgccccgccaccagcccaagccgcggcgagtcgtgcca aggtgtctcggaggtaccCCGCTCCCGTCTGGTCTCTACGTTTCGTGTACGGCCTGGATCCCGGAAAGG GCGGATGCACGTGGTGTGTCCTCCGCAATTGGCGCCACGTTTCAAAGTCCCGGCCAGAAAT GCACAGGACCGGCCCGCTCGCACAGGCCATGACGAATGCCAGATTTTCACAGCAAAACA ATCTGGAATAATCGCAACCATTCGCGTTTTGAACGAAACGAAAGACGCTGTTTAGCACGTTT

[illegible]

[0223] The construct can be written as *5'Thi4::PmHXT1v2:ScMEL1:PmPGK:CvNR:PmSAD2-2:NoLPAT3:CvNR::3'Thi4*. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from strain CHK22 that permit targeted integration at the *Thi4* locus via homologous recombination. The *P. moriformis* hexose transporter 1 (*HXT1 v2*) promoter driving expression of the *S. carlbergensis* melibiase (*ScMEL1*) is indicated by the boxed uppercase text. The initiator ATG and terminator TAA for *ScMEL1* are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. *P. moriformis* phosphoglucokinase (PGK) 3'-UTR is indicated by uppercase underlined text followed by a stearyl ACP desaturase-2 (*SAD2-2*) promoter from *P. moriformis*, indicated by the boxed italicized text, driving the expression of *NoLPAT3*. The initiator ATG and terminator TGA codons of *NoLPAT3* are indicated in uppercase, bold italics, while the remainder of the gene is indicated in bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the *P. moriformis Thi4 3'* flanking region indicated by bold, lowercase text.

[0224] Table 15 shows the fatty acid/TAG profiles of four primary transformants of CHK22 transformed with pCHK453. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 15: Screen of primary transformants of CHK22 with pCHK453.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK453 -1	0.03	1.20	26.34	3.37	60.29	6.78	0.48	0.26	0.10	1.49
pCHK453 -2	0.03	1.24	26.48	3.48	60.05	6.75	0.49	0.26	0.09	1.60
pCHK453 -3	0.03	1.20	26.28	3.07	60.33	7.09	0.47	0.24	0.07	1.57
pCHK453 -4	0.03	1.28	26.77	3.37	59.81	6.77	0.49	0.25	0.07	1.51
CHK22 Control	0.03	1.21	26.75	3.40	59.41	7.11	0.52	0.28	0.10	1.54

Example 9: Expressing *Volvox carteri* LPAAT2 (*VcLPAAT2*) in *P. moriformis* strain CHK22.

[0225] *V. carteri* *VcLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK785 contained 5' and 3' homology arms to permit targeted integration of *VcLPAAT2* into the genome and is shown in **Table 16**.

Table 16: Integrative sequences for the transformation of *P. moriformis* with pCHK785 encoding *VcLPAAT2*.

SEQ ID NO:	Sequence
8	gctcttcgcttgcgcgacccctcggtgatctgggagccctgcgcagcccttaaatcatctcagtcagggttctatgttcaactgagcctaaaggc ttcgtcacgcgacgagcacacgtatctcggtacgcagtcctctcagaagcggtagaaacagttcgcaagccctcgctcggtcgaaacttgcgcg agtactattgaattaaattaatgatcgaatgagacgcgaacttttgcagaatgccactgagtttgcagagaatgggagtgccgcatcacc atccgctgtgcacggcctgattcgccgagacgatgaatggcgagaccagggagcggttgcgagcccgagccggtagcaggaataacggtc gacaactcttctgccaattactggcaaccattagaaagagccggagcgcttgaaattctgcaatcgagtaatttttcgatgcggcggtcgt gaacctaaagctccgctatgtttaaggcgatcaagatgcacgcggcccgagcagtgctcgaagcacaacccagccttagttcgaga ctttgggagatagcggcagatatctagtttggcattttgtatattaattagctcgaatggagcgctctgatgcggtgcagcgtcggctgcagca cctggcagtggtgggtgcgcctatcgctcggcgccctggtcagctggctcccgccctcctcgcgcgccCTTTCTTGCGCTATGA CACTTCAGCAAAAGGTAGGGCGGGCTGCCGAGACGGCTTCCCGGCGCTGCATGCAACACCGA TGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCATTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCTCTTCGTTTCAGTACACAACCCGCAAACCTGTACAAATGctgtcaggcct tctgttctgtggtggcgttcgccccaagatcagcgctccatgacgaacgagacgtccgaccgccccgtgtgcacttaccccccaacagg ctggatgaacgacccccagcgctgtgtgacgacgagaaggacgccaagtggcaactgtacttccagtacaacccgaacgacacgtctggggga cgccctgttctggggccacgccacgtccgacgacctgaccaactggaggaccagcccatcgccatcgccccgaagcgcaacgactcggcgcc ttctcggctccatggtgtggactacaacaacacctccggtcttcaacgacaccatcgaccgcgccagcgctgcgtggccatctggacctaca caccccgagtcgaggagcagtagctctctacagcctggacggcggtacacettaccgagtaccagaagaaccccggtgctggcgccaact ccaccagttccgcgacccgaaggtcttctgtgacgacccctccagaagtggatcatgaccgcgccaagtccaggaactacaagatcgatct actctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggtctcctcggtaccagtacgagtgcccccgcgctgacgaggt ccccaccgagcaggacccagcaagtctactgggtgatgttcctcatcaacccggcgccccggcgcggtccttcaaccagtactctgctg gcagctcaacggcaccacttcgagcgcttcgacaaccagtcgccggtgggtggaacttcggaaggaactactacgccctgcagaccttctcaacac gacccgacctacgggagcgccctgggcatcgctgggctcccaactgggagttactccgcttcgtgcccaacccctggcgctcctcatgtccct

[illegible]

[0226] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:VcLPAAT2:CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text.

The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics.

The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *VcLPAAT2*. The initiator ATG and terminator TGA codons of the *VcLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics.

The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0227] **Table 17** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK785. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in the lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 17: Screen of primary transformants of CHK22 with pCHK785.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK785 -1	0.06	1.60	21.48	3.63	57.66	12.96	0.56	0.30	0.23	1.55
pCHK785 -2	0.06	1.68	24.57	3.76	55.08	9.77	0.42	0.16	2.02	1.58
pCHK785 -3	0.05	1.61	25.61	2.89	56.40	8.44	0.43	0.29	1.80	1.51
pCHK785 -4	0.07	1.78	22.51	3.73	53.75	11.69	0.46	0.33	2.88	1.55
pCHK785 -5	0.08	1.99	24.84	3.29	53.55	10.28	0.46	0.24	2.69	1.51
pCHK785 -6	0.06	1.77	25.64	3.32	55.68	9.90	0.48	0.11	1.38	1.52
pCHK785 -7	0.04	1.52	25.33	3.06	58.60	8.97	0.47	0.27	0.25	1.58

SEQ ID NO:	Sequence
	<i>gcaacctgtaaacaccgactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaataacaatca</i>GGCCGAC <i>AGGACGCGCGTCAAAGGTGCTGGTCTGTATGCCCTGGCCGGCAGGTCGTTGCTGCTGCTGGTTA</i> <i>GTGATTCGCGCAACCTGATTTTGGCGTCTTATTTTGGCGTGGCAACCGTGGCGCCCCGCGAGCCGG</i> <i>GCCGGCGGCGATGCGGTGCCCCACGGCTGCCGGAATCCAAGGGAGGCAAGAGCGCCCCGGGTCA</i> <i>GTTGAAGGGCTTTACGCGCAAGGTACAGCCGCTCCTGCAAGGCTGCGTGGTGAATGGACGTGC</i> <i>AGGTCTGCTGAAGTTCCTCCACCGCTCACCAGCGGACAAAGCACCAGGTGTATCAGGTCCGTGTC</i> <i>ATCCACTCTAAAGAGCTCGACTACGACCTACTGATGGCCCTAGATTCTTCATCAAAAAACGCTGAGA</i> <i>CACITGCCCAGGATTGAAACTCCCTGAAGGGACCACCAGGGGGCCCTGAGTTGTTCTTCCCCCCGT</i> <i>GGCGAGCTGCCAGCCAGGCTGTACCTGTGATCGAGGCTGGCGGGAAAATAGGCTTCGTGTGTCTCA</i> <i>GGTCATGGGAGGTGCAGGACAGCTCATGAAACGCCAACAAATCGCACAAATTCATGTCAAGCTAATCA</i> <i>GCTATTTCTCTTCACGAGCTGTAATTGTCCCAAAATCTGGTCTACCGGGGGTGATCCTTCGTGTA</i> <i>CGGGCCCTTCCCTCAACCCTAGGTATGCGCGCATGCGGTGCGCCGCGCAACTCGCGCGAGGGCCG</i> <i>AGGGTTTGGGACGGGCGCTCCCGAAATGCAGTTGCACCCGGATGCGTGGCACCTTTTTTGCATAA</i> <i>TTTATGCAATGGACTGCTCTGCAAAATCTGGCTCTGTGCGCCAACTTAGGATCAGCGGCGCTAGGAT</i> <i>TTCTGTAATCATTCTGCTCTGATGGGGAGCTACCGACTACCTAATATCAGCCCGACTGCCTGACGCCA</i> <i>GCGTCCACTTTTGTGCACACATTCCATTCTGTGCCCAAGACATTTTATTGTGGTGCGAAGCGTCCCCA</i> <i>GTTACGCTCACCTGTTTCCCGACCTCCTTACTGTTCTGTGCGACAGAGCGGGCCACAGGCCGGTTCG</i> <i>CAGCC</i><i>ATG</i><i>tgcgcgtaacgaaactctggtctgccctctctctgtttcagctttgttttactagtgteectacctcttggcattcttaaccgta</i> <i>tcgcttcgctcgtcgtggcaagcgcaacgacatgctgactggcccgctccctcgtggggtcttttgggtgacgcttttcaggcgagcgcaacag</i> <i>ggtctgtacaaggaggcccttgctatactgtgcaaccaccgctcttgggcccactctttgtcagcgctacctaaaggagggtcgcgtgctccctg</i> <i>atgagccgctggtgtgtactctgttccgggtgttctgtacctgtgcatgactcgaagggtatagtcctgtttaaagcggggacacatcgcggaaca</i> <i>ggagcgctttaaagcctggtggaacggacctgggtgactctcacgtccctggcctcctgtctaccggagggaacacggagcaaccaagcctacc</i> <i>tcgctgcccctgaagcgaggcatgctgactacgcgcattccgggaagctgcagtcaggctcatgctacgcgcggcaadagacgaggtcatgtccg</i> <i>agaaagcgagtccttcaacttcggcgcaactgctgctacgacattctcaaggctcctcaagccagcgactaccgaaacttgaggccttttgcgcg</i> <i>acctcaggctacgtggagcgctgctgggcagccacctacggcctgaaggacctgaaggcgctgcgcgctctcagctccctcccaggctta</i> <i>cactacagcgccaagacctgggcacagcaactggcgatcacgctcgtgctgactggttttgcggcgctgttaccgctcctggcggtggccttgc</i> <i>cgcagccctcgtgcacgggtgctgcgcaacaggtcggcgccagcaacagcaacagcaacagcgccgacggcgccctcagctcctct</i> <i>gggggatgggcccagggtcgcggcgccaggccgggggagcgctccagtggggacggggcgactggggcggtgggtggcgctgcccctgtcc</i> <i>ctggagggtgacgggtccttctgctgctgggtcggaggcagctgctcaggctcctctgTAG</i><i>tttaattaagcagcagcagctcgatagtatcga</i> <i>cacactctggagcgtgctgctgactgttgcggccacactgctgcttgacctgtgaataccttgcgcttttatcaaacagcctcagtggtttgat</i> <i>cttctgtgtacgcgcttttgcgagttgctagctgctgtctatttgcgaataaccacccccagcacccttccctcgtttatatacgttgcacccaaccgcaa</i> <i>cttatctacgctgctctgctatccctcagcgctgctcctgctcctgctcactgcccctgcacagccttgggttgggtccgctgtattctcctgctactgcaac</i> <i>ctgtaaacacgactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggaagcgtcgtgttgggagctggagctgctgggttgc</i> <i>gacggcgctgcagctcttcaggatgtgctggtgcgctgctgacgctgctgagaaatagtcgacgaaacgttgacggctggggccggc</i> <i>gggctgtggtatgcatacgcattgggtacgcggccattggatgggattggttaggcttatggagggaataagagtttttgcggatccaacgcgat</i> <i>ttgatgcagtagcccggtgtgctgaaagtatggaaggatgggtgctattgctatgcagctgcccaccccttttcgaggaaatgtgccgga</i> <i>tcgttgggtgacccatggggaaaatcgacgttcgaccactacatgaaaatgtataccttgaagatgcagcaactcggtgcaaacggataa</i> <i>cggtttggtaggtgtatgtcgcagcatgtgctggaatttgcgggcaactccccctgccacggccattgcaggtgtcatgttgactggaggatacga</i> <i>actttcggcgctcaaatccagaggaggacccctctggggcgacattgtgccactgctcttccgctgtgttctgtgtgaaattgt</i>

[0229] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:ChsLPAAT2:CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from

CHK22 that permit targeted integration at the DAO1b locus via homologous recombination,

the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene

(conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text.

The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while

the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is

indicated by uppercase underlined text followed by a linker. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *ChsLPAAT2*. The initiator ATG and terminator TGA codons of the *ChsLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0230] Table 19 shows the fatty acid/TAG profiles of six primary transformants of CHK22 transformed with pCHK786. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 19: Screen of primary transformants of CHK22 with pCHK786.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK786-1	0.06	1.64	22.11	3.43	57.43	12.61	0.55	0.32	0.26	1.56
pCHK786-2	0.06	1.63	21.58	3.52	56.71	14.04	0.55	0.04	0.27	1.99
pCHK786-3	0.06	1.80	26.04	2.82	55.77	9.53	0.57	0.07	1.61	1.50
pCHK786-4	0.04	1.48	25.14	2.93	58.91	9.00	0.49	0.25	0.25	1.58
pCHK786-5	0.04	1.47	24.92	3.10	59.00	8.98	0.48	0.29	0.27	1.50
pCHK786-6	0.06	1.60	21.73	3.35	57.88	12.90	0.55	0.04	0.31	1.60
CHK22 Control	0.04	1.52	26.43	2.97	58.76	7.88	0.44	0.25	0.23	1.53

Example 11: Expressing *Chlamydomonas incerta* LPAAT2 (*CiLPAAT2*) in *P. moriformis* strain CHK22.

[0231] *C. incerta* *CiLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK787 contained 5' and 3' homology arms to permit targeted integration of *CiLPAAT2* into the genome and is shown in Table 20.

Table 20: Integrative sequences for the transformation of *P. moriformis* with pCHK787 encoding *CiLPAAT2*.

SEQ ID NO:	Sequence
10	gctcttccgcttccccacacctggtgatctgggagccctgcgcagcccttaaatcatctcagtcaggtttctatgttcaactgagccctaaagggc tttctgacgcgcacgagcacagctatctcggtacgcagctctctcagaagcggtagaaacagtttgcgaagccctcgctcggtcgaaaacttgcgcc agtactattgaattaaatgaatgatcgaatgagacgcgaaacttttgcagaatgccactgagtttgcagagagaatgggagtgggcgccattcacc atccgctgtgacggcctgattcccgagacgatgaatggcgagacagggagcggcttgcagagccccgagccggtagcaggaataacggctg gacaactctctgtccaaactactggcaaccattagaaagagccgagcgcgttgaattctgcaatcagagtaattttctgatgcggcgggctctgt gaacctaaaggctccggcctatgtttaaggcgatccaagatgcacgcggccccaggcagctgtctcaagcacaacccccagccttagtttcgaga ctttgggagatagcggcagatatctagtttggcattttgtatattaaatagctcgcgaatggagcgtctgatcgggtgcagcgtcggctgcagca cctggcagtgggcgtgggctgcacctatcgtcgcggcctggtcagctggctcccgcctctcgcggcgccCTTTCTTTCGCTATGA <u>CAC'TTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCT'TCCCGGCGCTGCATGCAACACCGA</u> <u>TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGC</u> <u>GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCA</u> <u>TATTCAAACACCTAGATCACTACCACCTTCTACACAGGCCACTTAGAGCTTGTGATCGCACTCCG</u> <u>CTAAGGGGGCGCCTCTTCTCTTCGTTCTCAGTACAAACCCGCAAAGCTGTACAATG</u><i>ctgctgcaggcct</i> <i>tctgttctctgtgctggcggttcgcccgaagatcagcgctccatgacgaacgagacgtcgcagccgccccgtgtgcttaccaccaacaaaggg</i> <i>ctggatgaacgacccaacggcctgtggtacgacgagaagagcccaagtggcactgtatctccagtacaacccgaacgacaccgtctgggga</i> <i>cgcccttgttctggggccacgccacgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcc</i> <i>ttctcggctccatggtgtggtgactacaacaacacctccggcttctcaacgacaccatcgaccgcgcgcagcgtcgtggccatctggacctacaa</i> <i>cacccggaggtccgagggagcagtacatctctacagcctggagcggcgctacaccttaccgagtagaccagaagaaccccgtgctgcccgaact</i> <i>ccaccagttccgcgacccgaaggcttctgtgtacgagccctcccagaagtggatcatgaccgcggccaagtccaggactacaagatcgagatct</i> <i>actctccgacgacctgaagtctggaagtgtgctgcgttcgccaaaggcgcttcctgctgaccagtacgagtgccccgcctgcatcgaggt</i> <i>ccccacgacgaggaccagccaagctctactgggtgatgttacttccatcaaccccgcggcgccgctcttcaacagctactctgtcgc</i> <i>gcagcttcaacggcaccacttctgaggccttcgacaaccagctccgcgtgtgtgacttcggcaaggactactacgccctgcagaccttctcaacac</i> <i>gaccgacctacgggagcgccctgggcatcgctggggctccaaactgggagtactccgcttcgtgccaccaacccctggcgctctccatgtcct</i> <i>cgtgcgaagtcttcccaacacggagtagaccaggccaacccggagacggagctgatcaacctgaaggccgagccgatctgaacatcagaacg</i> <i>cgggccccgtgagccggcttcgccaacaacacacgtgtgacgaaggccaacagctacaacgtcgaacctgtcaacagcaccggcaccctggagtic</i> <i>gagctggtgtacgccgtcaacacacacacagacgatctccaagtctgtgttcgagacctctcctctgttcaaggcctggaggaccccgaggagt</i> <i>acctccgatgggttcgaggtgtccgctctctcttctgtgaccgcgggaacagcaagggtgaagttcgtgaaggagaacctacttaccac</i> <i>cgcattgagcgtgaacaacacagccctcaagagcagagaacgacctcttactactcaacaggtgtacggtctgtgacacgaacatctgagctgact</i> <i>taacgacggcgacgtcgtgtccaccaaacctacttcatgaccaccgggaacgcctgggtctcgtgaacatgacgacgggggtggaacacctgt</i> <i>tctatcagacaagttccaggtgcgcgaggtcaagtgaacTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCTCTCG</i> <u>AGGGCGCCTGTTCTCGCTTTCGCAAAACAAGCCCCCTGGAGCATGCGTGCATGATCGTCTCTGGCG</u> <u>CCCCGCGCGCGGTTTGTTCGCCCCTCGCGGGCGCGCGGCCGCGGGGGCGCATTGAAATTGTT</u> <u>GCAAAACCCACCTGACAGATTAGGGCCCCAGGACGAGGCGTTGAGATGGAGGTACAGGA</u> <u>GTCGAAGTAACTGAAAGTTTTTATGATAACTAACAAACAAGGGTTCGTTTCTTGCCACGCAATG</u> <u>ACAAGAACAAGATTCCACATTTCCGTGTAGAGGCTTGCCATCGAATGTGAGCGGGCGGGGCCG</u> <u>CGGACCCGACAAAACCTTACGACGTGGTAAGAAAAACGTGGCGGGGCACTGTCCCTGTAGCC</u> <u>TGAAGACCAGCAGGAGACGATCGGAAGCATCACAGCACAACCGGTgcagcagcagctcggataglatcga</u> <i>cacactctggagcgtgtgtgtgtgagctgttgcgccacactgtgtgcttgcactgtgaatatccctgcgcctttatcaaacgacctcagtggtttg</i> <i>atctgtgtgtacgcgttttgcgagttgtactgtgtgtgtctatttgcgaataacacccccagatcccccttctcgttccatctggttgcateccaaccg</i> <i>caactctctacgctgtctgtatccctcagcgtgtctctgtctctgtcactgtccccctgcacagccttgggttggctcgcctgtattctctgtgla</i> <i>gcaacctgtaaacacgacgactgtgatgcacgggaagttagtgggaacacaaatggacactagggaataacaatcaGGCCGAC</i> <u>AGGACGCGCGCTCAAAGTGTGCTGGTCTGTATGCTTGCCTGGCCGGCGAGGTCGTTGCTGCTGCTGGTTA</u> <u>GTGATTCCGCAACCTGATTTTGGCGTCTTATTTTGGCGTGGAACGCTGGCGCCCCGCGAGCCGG</u> <u>GCCGGCGGCGATGCGGTGCCCCACGGCTGCCGGAATCCAAGGGAGGCAAGAGCGCCCCGGGTCA</u> <u>GTTGAAGGGCTTACGCGCAAGGTACAGCCGCTCCTGCAAGGCTGCGTGGTGGAATTGGACGTGC</u> <u>AGGTCTGTGAAGTTCCTCCACCGCTCACCAGCGGACAAAGCACCGGTGTATCAGGTCCGTGTC</u> <u>ATCCACTCTAAAGAGCTCGACTACGACCTACTGATGGCCCTAGATTCTTCATCAAAAACGCCTGAGA</u> <u>CATTGCCCCAGGATTGAAACTCCCTGAAGGGACCAACAGGGGGCCTGAGTTGTTCTTCCCCCGT</u> <u>GGCGAGCTGCCAGCCAGGCTGTACCTGTGATCGAGGCTGGCGGGGAAAATAGGCTTCGTGTGCTCA</u> <u>GGTCATGGGAGGTGCAGGACAGCTCATGAAACGCCAACAAATCGCACAAATTCATGTCAAGCTAATCA</u> <u>GCTATTTCTCTTCACGAGCTGTAATTGTCCAAAATTTCTGGTCTACCGGGGGTGATCCTTCGTGTA</u> <u>CGGGCCCCCTCCCTCAACCTTAGGTATGCGCGCATGCGGTGCGCGCGCAACTCGCGCGAGGGCCG</u> <u>AGGGTTTGGAGCGGGCCGTCCCGAAATGCAGTTGACCCCGATGCGTGGCACCTTTTTTGCATAA</u> <u>TTTATGCAATGGACTGCTGTGCAAAATTCGTGCTGTGTCGCAACCCCTAGGCTACGCGCGTGAAGT</u> <u>TTGTAATCAITTCGTCTGTATGGGGAGCTACCGACTACCCCTAATATCAGCCCCGACTGCGCTGACGCCA</u>

SEQ ID NO:	Sequence
	<u>GCGTCCACTTTTGTGCACACATTCCATTTCGTGCCCAAGACATTTTCATTGTGGTGCGAAGCGTCCCCA</u> <u>GTTACGCTCACCTGTTTCCCGACCTCCTTACTGTTCTGTGCGACAGAGCGGGCCCCACAGGCCGGTTCG</u> <u>CAGCC</u>ATG<i>tcggctctgacgaaactctggggctccagcttctgttctcagttctgtgtttactggctcctgcgattttgccatctataccgc</i> <i>attcgctttgcgacctgggcaagcgcaacgacatgctggactgggcgcgcgcttagtcgggttttccgcgtgacgctgttacaggctggcgagca</i> <i>gagcctgtacaaaggggtcgtgctctacctgtgcaaccatcggtgtgggtgactctttatcagcgcctacgtgacggaggccggggcggc</i> <i>tgatgtcgcgtggctcgtgactttgtcttctgttctgcacctctgcatggtcttgaagggtcgtcgttcaagcgcggtacaatcgggacaa</i> <i>ggaggccttcaacgcttggctgacgggacctcctggcgacagccatgtgccggcctcctggctacccggaggacacgcctccaccaagcctgc</i> <i>gtactgcgcgtcaagaggggtgctgctactacgctactccgcaagctccgggtgcaggctattatcacaggggttaaggacgaggtcctgagt</i> <i>gagaagtcaccaatcggtgacttcggcgacgtgctgacgacttttgaagggtatcaagccctcggaacttcccaacttcgaggcgttcttgc</i> <i>gatctccaggcaacctgggacgcgggtggacggccactacggcctgaaggacctcaaggcgctcccccgtattctccatgccagcccgaggc</i> <i>gtacacgtactcaagcaagatgtggcgacgcaactggcgatcacccctttcagcattccggcttttcaggcgctgctacggtagctggcgctgtt</i> <i>gctgccgcgtggctgccacgggtgccgtcaacaggtgggtacacctcgccttagctgggtgggtggaggctcggtgctcgaggagctttctg</i>TAG<i>t</i> <i>taattaagcagcagcgtcggatagatcgacacactctggacgctgtgctgtgactgttgccgccacactgctgcttgaacctgtgaataccct</i> <u>cccgtttatcaaacagcctcagtgctttgatctgtgtgacgcgcttttgcgagttgctagctgcttgcgaataaccacccccagcacccttc</u> <u>ctcgtttcatatcgcttgcacccaaccgcaacttatctacgctgctcctgctatccctcagcgtgctcctgctcctgctcactgccctcgcacagccttgggt</u> <u>tggctccgcctgtattctcctgtgactgcaacctgtaaacgacgactgcaatgctgtatgcacgggaagtatgggtgggaacacaaatggaagcgtct</u> <u>gcgtgttgggagctggagtcgtgggtgacgacggcgctgacgctcttgcaggatgtgctggcgctgcggttcacgtgctgctgagaaatag</u> <u>gcgacgaaacgttgacggctggggccggcggtgtggtatgccatacgcatgggtacggccattggatgggattggtagcttattggagg</u> <u>ataatagatgttttcggatccaacgcatgttgatgcagtagcccggtgtgctgaaagtatggaaggatgggtcattggctattcacatgcgctgc</u> <u>ccaccccttttcgaggaaatgtccggcatcgttgggtgcacgatggggaaatcgacgttcgaccactacatgaaatgtataccttgaagat</u> <u>gcagcaactgcgggtgcgaaacggataacggttggtaggtgtatgtgcagcatgtgctgattttgcgggcaactccccctgccacggccatt</u> <u>gcagggtgcatgttactggaggatacgaacttccggcgtcaattccagaggaggaccccccttgggcccgaacttgcgccactgctcttccg</u> <u>cttgcgtttcctgtgaaattgt</u>

[0232] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*CiLPAAT2*:CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *CiLPAAT2*. The initiator ATG and terminator TGA codons of the *CiLPAAT2* are indicated in uppercase, bold italics, while the remainder of the gene is indicated in bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0233] **Table 21** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK787. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in the lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct

transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 21: Screen of primary transformants of CHK22 with pCHK787.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK787-1	0.06	1.59	21.37	3.80	56.59	13.98	0.55	0.27	0.27	2.13
pCHK787-2	0.05	1.55	21.51	3.25	57.93	12.91	0.61	0.30	0.28	1.55
pCHK787-3	0.06	1.56	22.21	3.60	57.26	12.69	0.52	0.27	0.28	1.95
pCHK787-4	0.04	1.46	25.58	3.15	58.81	8.53	0.46	0.26	0.25	1.59
pCHK787-5	0.06	1.54	21.36	3.69	57.17	13.54	0.53	0.29	0.30	2.21
pCHK787-6	0.06	1.55	22.40	3.84	57.60	12.16	0.51	0.03	0.29	2.04
pCHK787	0.05	1.65	23.19	3.06	57.86	11.70	0.56	0.04	0.26	1.53
CHK22 Control	0.04	1.52	26.43	2.97	58.76	7.88	0.44	0.25	0.23	1.53

Example 12: Expressing *Volvox africanus* LPAAT2 (*VaLPAAT2*) in *P. moriformis* strain CHK22.

[0234] *V. africanus* *VaLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK788 contained 5' and 3' homology arms to permit targeted integration of *VaLPAAT2* into the genome and is shown in **Table 22**.

Table 22: Integrative sequences for the transformation of *P. moriformis* with pCHK788 encoding *VaLPAAT2*.

SEQ ID NO:	Sequence
11	gctcttcgctgcccgcacccctggtgatctgggagccctgcgcagcccttaaatcatctcagtcagggttctatgttcaactgagcctaaagggc tttcgtcacgcgcagcagcacagctatctcggtacgcagctctctcagaagcggtagaacagttcgcaagccctcgctcggtcgaaaaacttgcgcc agtactattgaattaaattaatgatcgaaatgagacgcgaaacttttcagaatgccactgagtttgccagagaatgggagtgccgcattcacc atccgctgtgcacggcctgattcgccgagacgatgaatggcgagaccaggagcggttcgagagcccgagccggtagcaggaataacggtc gacaatcttctgtccaattactggcaaccattagaaagagccggagcggttgaattctgcaatcgagtaatttttcgatcgccgggctgct gaaccctaaggtctcgccctatgtttaaggcgatccaagatgcagcggcccccaggcagctgtctcaagcacaaacccagccttagtttcgaga ctttgggagatagcggcagatatctagtttggcattttgtatattaagttctcgcaatggagcgctctgatcggttcagcgtcggttcgagca cctggcagtgccgtgggtcgccctatcgctcgccgctggtcagctggtctccgctctctcgccgcgcgcCTTCTTGCGCTATGA CACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAACACCGA TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCCTCTTCGTTTCAGTCACAACCCGCAAACCTGTACAATGctgctgaggcct tctgttctgtggtggcgttcgcccaagatcagcgctccatgacgaacgagacgtccgaccgccccctggtgcacttcccccaacaagg ctggatgaacgaccccaacggcctgtgtgtacgacgagaaggacgccaagtggcactgtacttccagtacaacccgaacgacacgtctggggga cggccctgttctggggccacgacgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactcggcgcc ttctccgctccatgggtgggactacaacaacacctccggcttctcaacgacaccatcgaccgcgccagcgtgcgtggccatctggacctaca

[illegible]

SEQ ID NO:	Sequence
	gagttttgtcggatccaacgcatgttgatgcagtagcccggtgtgctgaaagtatggaaggatgggtgcattggctattcacatgcgctgccacccc cttttcgcaggaaatgtgccggcatcggtgggcaccgatggggaaaatcgacgttcgaccactacatgaaatgtatacctctgaagatgcagc aactcgggtgcgaaacggataacggttggtaggtgtatgtcgcagcatgtgctggatttgcggggcaactccccctgccacggcccaatgcagg tgtcatgttactggaggatacgaacttcggcgcgtaaatccagaggaggacccccctctgggcccacattgtgccactgctctccgcttct gttctctgtgtaaattgt

[0235] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:VaLPAAT2:CvNR::3'DAO1b.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *VaLPAAT2*. The initiator ATG and terminator TGA codons of the *VaLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *C. vulgaris* nitrate reductase 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0236] **Table 23** shows the fatty acid/TAG profiles of six primary transformants of CHK22 transformed with pCHK788. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in the lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 23: Screen of primary transformants of CHK22 with pCHK788.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK788-1	0.05	1.60	22.85	3.53	57.65	11.64	0.50	0.29	0.28	1.76
pCHK788-2	0.05	1.55	21.80	3.70	57.40	12.85	0.53	0.29	0.28	1.76
pCHK788-3	0.05	1.61	23.72	3.19	57.12	11.72	0.54	0.04	0.39	1.96

pCHK788-4	0.06	1.62	21.68	3.88	56.61	13.71	0.55	0.03	0.31	1.96
pCHK788-5	0.05	1.58	24.35	3.27	57.83	10.37	0.47	0.29	0.27	1.68
pCHK788-6	0.06	1.55	21.86	3.61	57.60	12.88	0.52	0.04	0.31	1.86
CHK22 Control	0.04	1.52	26.43	2.97	58.76	7.88	0.44	0.25	0.23	1.53

[0237] As the transformants of CHK22 expressing CrLPAAT2 produced the higher OP:OO ratio, the percent identity (%) of all the amino acid sequences across the entire LPAAT protein was compared against that of CrLPAAT2. The results are shown in **Table 24**. The resulting phenotype of expressing these LPAATs in CHK22 is indicated with the m/z (577.5/603.5) OP:OO ratio; the OP:OO ratio shown in the rows of PmLPAAT1 or PmLPAAT2 represents the analytical results of the non-transgenic line CHK22.

[0238] In some embodiments, a microalgal cell provided herein comprises an exogenous gene that encodes for an LPAAT in **Table 24**. In some embodiments, the exogenous gene can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a LPAAT in **Table 24**.

Table 24: OP:OO Ratio

Gene Name	Organism	GenBank ID	Identity (%)	m/z (577.5/603.5) OP:OO
CrLPAAT2	<i>Chlamydomonas reinhardtii</i>	XP_001699586.2	100.0	3.45
CiLPAAT2	<i>Chlamydomonas incerta</i>	KAG2426747.1	89.8	2.21
ChsLPAAT2	<i>Chlamydomonas schloesseri</i>	KAG2454274.1	77.2	1.99
VaLPAAT2	<i>Volvox africanus</i>	GIL63906.1	69.2	1.96
NoLPAT3	<i>Nannochloropsis oceanica</i>	BAW99691.1	7.6	1.6
SII1848	<i>Synechocystis sp</i>	WP_010873224.1	17.7	1.60
CrLPAAT1	<i>Chlamydomonas reinhardtii</i>	XP_042921325.1	15.5	1.59
EcPlsC	<i>Escherichia coli</i>	HBN6599230.1	10.5	1.59
VcLPAAT2_98847_V1	<i>Volvox carteri_98847</i>	XP_002957774.1	47.5	1.58
BnBAT2	<i>Brassica napus</i>	NP_001302928.1	14.1	1.57

Gene Name	Organism	GenBank ID	Identity (%)	m/z (577.5/603.5) OP:OO
NoLPAT4	<i>Nannochloropsis oceanica</i>	BAW99690.1	11.2	1.55
AtLPAAT2_ABG48392	<i>Arabidopsis thaliana_ABG48392</i>	NP_567052.1	11.7	
BnBAT1.13	<i>Brassica napus_ADC97478</i>	NP_001302981.1	11.7	
BnBAT1.5	<i>Brassica napus_ADC97479</i>	NP_001302955.1	10.5	
CnLPAAT2	<i>Cocos nucifera</i>	Q42670.1	14.4	
CosLPAAT2	<i>Coccomyxa subellipsoidea</i>	XP_005647638.1	47.2	
CuPSR23_LPAAT2-1	<i>Cuphea PRS23</i>	LG018472.1	12.2	
CuPSR23_LPAAT3-1	<i>Cuphea PRS23</i>	LG018473.1	13.3	
CuPSR23_LPAATx	<i>Cuphea PRS23</i>	LG018471.1	16.2	
LaLPAAT2	<i>Limnanthes alba_AAC49185</i>	Q42868.1	13.2	
MaLPAAT2	<i>Mortierella alpina_AED33305</i>	XP_002501997.1	9.383	
MpLPAAT2	<i>Micromyces pusilla_XP_002501997</i>	XP_002501997 .1	9.4	
OILPAAT2	<i>Ostreococcus lucimarinus</i>	XP_001421357.1	32.8	
ScSLC1	<i>Saccharomyces cerevisiae</i>	NP_010231.1	13.2	
PmLPAAT1	<i>Prototheca moriformis</i>	N/A	8.4	1.54
PmLPAAT2	<i>Prototheca moriformis</i>	N/A	32.1	1.54

[0239] Table 25 shows the amino acid sequences of LPAATs described in the Examples herein.

FIG. 3, Panels A-C illustrate amino acid sequence alignments of the amino acid sequences of LPAATs described herein, including EcPlsC (SEQ ID NO: 22), OILPAAT2 (SEQ ID NO: 63), PmLPAAT2 (SEQ ID NO: 24), CosLPAAT2 (SEQ ID NO: 64), CrLPAAT2 (SEQ ID NO: 13), ChsLPAAT2 (SEQ ID NO: 14), CiLPAAT2 (SEQ ID NO: 15), VaLPAAT2 (SEQ ID NO: 21), VcLPAAT2 (SEQ ID NO: 20), NoLPAT4 (SEQ ID NO: 17), NoLPAT3 (SEQ ID NO: 16),

PmLPAAT1 (SEQ ID NO: 23), Sll1848 (SEQ ID NO: 19), BnBAT2 (SEQ ID NO: 18), and CrLPAAT1 (SEQ ID NO: 12). The consensus sequence is reflected in SEQ ID NO: 157.

[0240] In some embodiments, a microalgal cell provided herein comprises an exogenous gene that encodes for an LPAAT in **Table 25**. In some embodiments, the exogenous gene can comprise a sequence that encodes for an enzyme with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a LPAAT in **Table 25**. In some embodiments, the exogenous gene can comprise a sequence that encodes for an enzyme with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 12-24, 63, and 64.

Table 25: LPAAT Amino Acid Sequences

SEQ ID NO:	LPAAT	Sequence
12	CrLPAAT1	MARKSSLAQA AIERKPVLLRPQLNVPRMSGITALPMERRPLPVA PSPSAKPELPARSALVCHAAAASVPLPNSDSAPQPNVLLAKIRAI MFFAWSFLLSLPLFVTMMVMAPLVLAFDKYRRLAQHFVNNLW ACASTAPFYKVTIIGRENLPDPKPVVYVANHQSFLLDIYSLFHLQ RPFKFISKTSNFLIPIGWSMFLTGHVMINRVDRRSQKCLQCCR DLLAEGAPVLFFPEGTRSLDCKMAGFKKGAFSVAAGVEVVP TLLGTGSLMPSGKESQLRPGQVTIVVHKALPPNKNADQLCDAA RQAVASSLPPELVGSATEMAPDEQ
13	CrLPAAT2	MSVLTKWLGLPSFLFSVFVFWSLPIFAILYRIRFASLGKRNDML DWARALVAYFRVTLLQAGEHTLYKGGPCLYLCNHRSWADFFID AYLTEGRAALMSRWLVYFVFPVFCTSCMILKGIVLFKRGTIADK EAFNAWLDQTLGSSHVPGLLVYPEGHRSTKPASLPLKRGMLHY AHSRKLVPQIVVTRGKDEVLSEKSQSVHFGRTCVTTFKSVLKSA DYPNFEAFFTDLQATWDSCWAATYGLEDLKNVPRFSMPGPQAY SYSSSMWVQQLAITLVLSILVFAGVCYGSWRGLAAALAAATGAAQ QVVALVLAAWVGSSVLRSL
14	ChsLPAAT2	MSALTKLLGLPSFLFSVFVFWSLPIFAIFYRIRFASLGKRNDML DWARSLVGFFRVTLQAGEQGLYKGGPCLYLCNHRSWADFFV DAYLTEGRAALMSRWLVYFVFPVFCTSCMILKGIVLFKRGTIAD KEAFNAWLDRTLGD SHVPGLLVYPEGHRSTKPTSLPLKRGMLH YAHSRKLVPQVIVTRGKDEVMSEKQSVHFGRTCVTTFKSVLK PADYPNFEAFFADLQATWDACWAATYGLKDLKGVPRFSMPSPQ AYTYSAKTWAQQLAITLVSIIVFAAVCYGSWRGLAAALAAATGA AQQVRGQQQQQQQQRRRLSSSGGWAEGRGAQAGGRLQWD GGDWGRWVALPLSLEVTVLLAAWVGGSVLRSL
15	CiLPAAT2	MSALTKLLGLPSFLFSVFVFWSLPIFAILYRIRFASLGKRNDML DWARALVGFFRVTLQAGEQSLYKGGPCLYLCNHRSWADFFID AYLTEGRAALMSRWLVYFVFPVFCTSCMV LKGIVLFKRGTIAD KEAFNAWLDRTLGD SHVPGLLVYPEGHRSTKPASLPLKRGMLH YAHSRKLVPQVIITRGKDEVLSEKSQSVHFGRTCVTTFKSVIKPS DFPNFEAFFADLQATWDAGWTATYGLKDLKGVPRFSMPSPQAY

SEQ ID NO:	LPAAT	Sequence
		TYSSKMWAQQLAITLFSIPVFAGVCYGSWRVLAAALAATGAAQ QVVTALAGWVGGSVLRSL
16	NoLPAT3	MWGLLWKHMTLRLLFVSVCLVTSSIGANMMALALFLVIRPFSR SLYRRLVSQCVACMWIDALSLLLPGTNIHIAADSMDPDGITAGIV VANHQYEGDWWFMLMVARFLGLHGNVKIIVREGLKKIPLLGW LVRLVEYPFISSWSLSRTNLFGLLRSFNADDFPVLLFQFPEGDRI DAKVRQQSLAFAAKEQRPHLLHVLLPRTTGFNTCIEALRTSHPP VYDMTIAIPGTTGQPSSSSSSSSPSAAAAAASPSAGAAAAAAAAS NTSTNPSSNPSSSSSTDTSPAAAAAAAADAAAAESHDAASFHTFL RFCNGEGPRDVHIRLKRYSLNDVLADPHWLDTKWAEKDRVLT YFSRHACFPAPLPPHMANGGGSTQTHGGRGGPRGPAGSHAVVG AAGNAQYLRSFNRSRKFAETSFLALARLLLTPCLPLLLLMACP LMTLYVCVSTVRRWLGEIIVFSPGGREGGREGDAVYESVNWSV
17	NoLPAT4	MSGPADARGPARVSDTIIGAPDRHHVGLTILVALLSAFCFCEFF MLPVAMALLPVPGTTSRKLRRWEGLVAKTWLSFGGWLLENV GGVKLILTGDSILPGDRVLILSNHRTRIDWMFLWCWAARFDLLS SYRVILKSSLRTFPWWGWGMSLCLFPFIHRGSNNRDADLRIEHI CQYLSELGVPNSLILFPEGTDLSQSNQKRDRDYALAKNLPYHN VLHPRSGAFIASLTAMRPHLDAVVDLTIGYVDYTPGERPSELSLL KGRLPHEVHINMKRWDIKTTPLLRENPSDRAEQFLRESFDRKEA LLTAFYSNNTDTSGGGLKQTSSLLVSSSSSPFSNSSAPLGFPSPSSP STSVASSYNPVPVYVHNAHWTYGKGLLGTGIAMALVLMACCLP AVAFCGYSIVVFLFVTVNSVWEGFDTLELNLAVWWGGQAGQ TKQE
18	BnBAT2	MDVASARGVSSHPPYYSKPICSSQSSLIRIPISKGCCFARSSNLITS LHAASRGVTRRTSGVQWCYRSIRFDPFKVNDKNSRTVTVRSDLS GAATPESTYPEPEIKLSSRLRGICFLVAGISAIVLIVLMIIGHFPVL LFDRYRRKFHHFIAKLWASISIYPFYKTDIQGLENLPSSDTPCVYV SNHQSFLLDIYTLSSLGQSYKFISKTGIFVIPVIGWAMSMMGVVP KRMDPRSQVDCLKRCMELVKKGASVFFPEGTRSKDGRGLGPFK KGAFTIAAKTGVPVPITLMGTGKIMPTGSEGILNHGDVRVIIHK PIYGSKADVLCEEARNKIAESMNLLS
19	SII1848	MDSEINHRGGLSAPRPRETSLNLALYRGLKWGVVRPLLHGLFQ AQVYGQELVPTRGPALVVSNHASYFDPPFLSCAMARPVAFMAK EELFNVPLLGPAILRYGAYPVKRGSGDRGALRAALTALGDGWL VGVFLEGTRTKDGRIHQPKLGAAMIAAKAQVPIIPVSLGGVEQIF QPGSPWPHPVPLTIRIGKAIAPPVKNRKPELEAVTKACQAQIHEM LDLGRD
20	VcLPAAT2	MSVPSKALSPLSFLFSVFVFWSLPLFAIIYRIRFANVGKRNDML DWWMVFFAFPVFCTSVLILRGIVLFKRGHIADKELLKSDAVVV MVVMVATVVQAFNVWLDATLRKSTVPGLLVYPEGHRSLKPR SLPLKRGMLHFAFSRHLAVQLVITRGKEEVMSEKTC SARWGRT LVTNYSKVCAGGRWGGVIKPTDFDSFDAFFNEIQTWDACWQL SYHQPIAGVPRLSLKDAHEYDYPPSMRWLQLGVTLISIVILAAV LYGSWVVLRAVLSLGSSTVQQAVVVLGGAGGWLGLSLLRSFL
21	VaLPAAT2	MSVIRKALSPLSFLFSVFVFWSLPFFAIIYRIRFINVGKRNDMLD WAKALVAFFHVTVLKQGEMTLYRGGRCLYLCNHRSWADFFID TYLTEGRAALMSRWLVYFVFPVFCSSVIVLRGIVLFKRGHIANK

SEQ ID NO:	LPAAT	Sequence
		EAFNAWLDVTLGKSTVPGLLVYPEGHRSLKPTSLPLKRGMLHF AYSRLPVQIIVTRGKEEVLSEKTL SARWGRTL VVTFSHVLKPSD YDSFDAFFDDIQHKWDACWKSSYDKPTAGVPELSMKGAHEYD YPRSMWWLQLGMTLMSIVILIGVMWGSWVALRTGLAVLGAAQ QPVVLC AAVWIGASMLRAFF
22	EcPlsC	MLYIFRLIITVIYSILVCVFGSIYCLFSPRNPKHVATFGHMFGR LAP LFGLKVECRKPTDAESYGNAIYIANHQNNYDMVTASNIVQPPTV TVGKKSLLWIPFFGQLYWLTGNLLIDRNNRTKAHG TIAEVVNH KKRRI SIWMFPEGTRSRGRGLLPFKTGAFHAAIAAGVPIIPVCVST TSNKINLNLRLHNGLVIVEMLPIDVSQYGKDQVRELA AHCRSIM EQKIAELDK EVAEREAAGKV
23	PmLPAAT1	MAREGLDSVETADGGAKPENGAGEDVREGRSSPSDEDEALPAE LEGLYPEAPIHGPDAGFAERVMTLQAKHDLAGKGDALLKGT LEETTQLRLRKG EAGSEAGPAGSGSLYELARSSPFIDLQHPIDAY ELAKMVFLAPLFLFRALILLVLVASAYLSVMVMFHVLPAGAHG LYPGRDALAQLLIHRCCPILLFSLSGFYHIPIKGAENIQKAKEARSI VIFNHLSYVDP AVLCLMPLSGIAKAGVARIPFIGPLGVALRWLF VERRGSSDRSNPHTLKGDPIDAIRARRADHSAPLLVMAPEATTK AQQCLLKFRRGAF TSGCPVTPVLIRYFRHFNPAGWGLCRSTPLH VYRLLVQPVNHVSLEVLPPYFPDEQETKDWTRFASNVRVRMAQ ALQAPMVEQGIAEERRLLKAGVTTDLAGSRIIFRRKVAEQATSP RKRD
24	PmLPAAT2	MTLAASIKLALRWPLFLLSMALLYWSVVVGS LGGYIKFLDLKSE QRGDLFAWSVAIKQAFGIRVLRVGKRDVYRGKCMYLVSWGM MAVVFP LFMIALRAMRAVLVFN RGQIPDKEKFNNWIDKELANT PQTGLAVYPEGHRSVLSDSLPLKRGMLHYAYS RKMTVQCIICAN KEAVLSEKYQVARWGQTVWAGYSDPINAADFDSFEAFMTTVQ STWDTLWKEVYGADPTGLPDLEMDDPVLDTPAHLRYAVSASV VLVDVVFFLV LWFGARFAIWATSPLGPLRGYLLFAAILTVVASF VVYSEPFNSVAYNARKHQRRAAADPNRVRATGASISDKPAGVA PPTDLVAHSVKDVTGDSLLVDGELDSKKDA
63	OILPAAT2	MAPVFALSFQLSIFASTD GARVDRRARRSPGFEWGKFMTRSVAN ARFKLAPGSGTLAREKRRTVWLCNHRAWSDFFLDIYLTEGRAFT MSRMLVAYAFPLFMVPAMVCGVVFGFKRDAGDKEKLNAALD AHFDAFGGEFAGMVCYPEGTRNVRENSMPLKRGMLRYAHSRK MAVQCVCTARKERV FSSHLTCAERGLALPVYFAEVIHAEDYPDF EDFYNEIRSRWDNAWSAANGVSAEAEESLPDYTPREHVVRCTP RHDAVIVSSALLTVLGVALTWRA CSKLL
64	CosLPAAT2	MGVLEKIQTLP AFFLG VFFLYWSLPLAALMNKV KFLKLAGKRN DAYGWGVALQQLFGVTMLRLPGPQLANPCGP IIFLSNHRSWAD FYLDVVATEGNAQMLSRMAVALVFP MFMGAVCVIRSVILFQRG RNHEKEAFNNMIDRKMAASPVDGLIVYPEGHRSTLRHSLPLKRG MLNYAYIRKIPVQVIFCNAVLC LL

Example 13: Expressing *Volvox carteri f. nagariensis* LPAAT2 (VcLPAAT2 V2) in *P. moriformis* strain CHK22.

[0241] *VcLPAAT2* V2 was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK967 contained 5' and 3' homology arms to permit targeted integration of *VcLPAAT2* V2 into the genome and is shown in **Table 27**.

Table 27: Integrative sequences for the transformation of *P. moriformis* with pCHK967 encoding *VcLPAAT2* V2.

SEQ ID NO:	Nucleotide Sequence
25	gctcttcgcttgcggcaccctcgttgatctgggagccctgcgcagcccttaaatcatctcagtcaggtttctatgttcaactgagcctaaagggtttcgtc acggcagcagcacacgtatatacggctacgcagtcctcagaagcggtagaacagttcgcaagccctcgtcggcgaaaacttgcggcagctactattgaat taataataatgatcgaatgagacgcgaaacttttcagaatgccactgagtttcccagagaatggagtgccgccattaccatccgcctgtgcacggcct gattcggcagacgatgaatggcgagaccaggagcggttcgagccccgagccggtagcaggaataacggcgcacaatcttctgtccaactactgg caaccattagaaagagccggagcgcgttgaaattctgcaatcgagtaattttcgtatgcggcgccctgctgaacctaaaggctccggcctatgtttaaggc gatccaagatgcacggcgccccaggcacgtgtctcaagcacaacccagccttagtttcgagactttgggagatagcggcagatatactagtttggcatttt gtatattaataagtcgcgaatggagcgtctgatcggtgcagcgtcggtgcagcacctggcagtgccgctggggcgcctatcgtcggcgccctgg tcagctggcctccgcctcctgcggcgcccdCTTTCTTGGCGCTATGACACTTCCAGCAAAAGGTAGGGCGGGCT GCGAGACGGCTTCCCGGCGCTGCATGCAACACCGATGATGCTTCGACCCCCCGAAGCTCCTTC GGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCC GATTGCAAAGACATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCCT TCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCTCGTT TCAGTCACAACCCGCAAACTGTACAATGctgtgcagcgcttctgttctgtcgtggcggttcgcccgaagatcagcgccctc catgacgaacgagacgtccgaccgcccctgtgtcacttaccaccaacaaggcggtgatgaacgaccaacggcctgtgtgacgagagaaggac gccaagtgtgcacgtgacttccagtacaacccgaacgacaccgtctgggggacgcccctgttctggggccacgccagtcgcgacgactgaccaactgg gaggaccagccatcgccatcgccccgaagcgcaacgactcggcgcccttcggctccatggtgtggtactacaacaacacctccggcttcttaac gacaccatcgaccgcggcagcggtgcgtggccatctggacctacaacaccccgagtcggagcagctacatctctacagcctggacggcgccga caccttaccgagtagcagaagaaacccgtgctggcgccaaactccaccagttccgcgaccgaaggttcttggtagcagcctccagaaagtgat catgaccgcggccaagtccagactacaagatcgagatctactctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggcctt cctggctaccgtacgagtgccccggcctgacgaggtcccaaccgagcagggacccagcaagtcctactgggtgatgttatctccatcaacccccgc gccccggcgcggtcctctcaaccagtacttctgcggcagcttcaacggcaccacttcgagggccttcgacaaccagtcctccgctgtgtgacgttcggca aggactactagccctgcagaccttcttaacaccgaccgacctacgggagcgccctgggcatcgctggcgctcctaactgggagtagtaccgcttcgt gccccaccaacccctggcgctcctccatgtccctgtgcgaagttctccctcaacaccgagtagcagggccaacccggagacggagctgatacaactgaa ggccgagccgatcctgaacatcagcaacggcgccctggagccggttcgccaccaacaccagttgacgaaggccaacagctacaacgtcgaactgt ccaacagcaccggcaccctggagttcgagctggtgtacgccgtcaacaccaccagacgatctccaagtcgttcgcggacctctccctctgtttcaa gggcttggaggaaccccgaggagtagctccgcatgggcttcgaggtgtccgcgtcctcttcttctgaccgcgggaacagcaaggtgaagttcgtgaa ggagaacccctacttcaacacgcgatgagcgtgaacaaccagccctcaagagcgagaacgacctgtcctactacaaggtgtacggctgtctggacca gaacatctggagctgtacttcaacgacggcgacgtcgtgtccaccaacacttactatgaccaccgggaacgcccctgggtccgtgaacatgacgacg gggtgtgacaacctgttctacatcgacaagtccaggtgcgcgaggtcaagtgactTAAGACGCCCGCGCGCGCACCTGACC TGTTCTCTCGAGGGCGCTGTTCTGCTTGGCTTGGCAAAACAAGCCCCCTGGAGCATGCGTGCATGATC GTCTCTGGCGCCCCGCCGCGCGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGCGCATT GAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGTTGAGATGGAG GTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAAGGGTCGTTTCTGGCC AGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGCTTGGCATCGAATGTGAGCGG GCGGGCCGCGGACCCGACAAAACCTTACGACGTGGTAAGAAAAACCTGCGCGGCGCACTGTCC CTGTAGCCTGAAGACCAGCAGGAGACGATCGGAAGCATCACAGCAACCGGtgacgacgagctcg gatagatcgacacactcggacgctggtcgtgtgactgttgcggccacactgtcgtccttgacctgtgaatfatccctgccgttttatcaaacagcctc agtgtgttgatctgtgtacgcgcttttgcgagttgtagctgctgtgtatttgcgaataccacccccagcatcccttccctctttcatatcgttgatc ccaaccgcaacttatctacgtgtcctgtatccctcagcgctgtcctgtcctgtcctactgcccctcgcacagccttggttgggtccgcctgtatttctct ggtactgcaacctgtaaaccagcactgaatgctgatgcacgggaagtagtggaaggaacaaatggacctaggaaataacaatcaaatgcttGGC CGACAGGACGCGCGTCAAAGGTGCTGGTTCGTGTATGCCCTGGCCGGCAGGTTCGTTGCTGCTG CTGGTTAGTGATTCCGCAACCCTGATTTTGGCGTCTTATTTTGGCGTGGCAACCGCTGGCGCC CGCGAGCCGGGCGCGCGGCGATGCGGTGCCACGGCTGCCGGAATCCAAGGGAGGCAAGA GCGCCCCGGGTCAAGTTGAAGGGCTTTACGCGCAAGGTACAGCCGCTCTCTGCAAGGCTGCGTGG TGGAATTGGACGTGCAGGTCCTGCTGAAAGTTCTCCACCGCCTCACCAGCGGACAAAGCACC GGTGTATCAGGTCCGTGTCTACTCTAAAGAGCTCGACTACGACCTACTGATGGCCCTAGA TTCTTCATCAAAAACGCCTGAGACACTTGCCAGGATTGAAACTCCCTGAAGGGACCACCAG

SEQ ID NO:	Nucleotide Sequence
	GGGCCCTGAGTTGTTCCCTCCCCCGTGGCGAGCTGCCAGCCAGGCTGTACCTGTGATCGAGGCTGGCGGGGAAAATAGGCTTCGTGTGCTCAGGTCATGGGAGGTGCAGGACAGCTCATGAAACGCCAACAAATCGCACAAATTCATGTCAAGCTAATCAGCTATTTCCCTTCACGAGCTGTAATTGTGCCAAAATTCTGGTCTACCGGGGGTGATCCTTCGTGTACGGGCCCTTCCCTCAACCCTAGGTATGCGCGCATGCGGTGCGCGCGCAACTCGCGCGAGGGCCGAGGGTTTGGGACGGGGCCGTCCCGAATGCGAGTTGCACCCGGATGCGTGGCACCTTTTTTGGCGATAATTTATGCAATGGACTGCTCTGCCAAAATTCTGGCTCTGTGCGCAACCCTAGGATCAGCGGCGTAGGATTCGTAATCATTCTGTCCTGATGGGGAGCTACCGACTACCCTAATATCAGCCCGACTGCCTGACGCCAGCGTCCACTTTTGTGCACACATTCCATTTCGTGCCCAAGACATTTTCATTGTGGTGCGAAGCGTCCCCAGTTACGCTCACCTGTTTCCCGACCTCCTTACTGTTCTGTGTCGACAGAGCGGGCCCCACAGGCCGGTTCGACGCCGTACATGagcgctccctcgaaggccctgcctctgcctcgtttctgtctctgttctgttctactgtgcgctgcctttgcgattatctaccgtatcgcttcgccaaagctgcgcaagcgcaacgacatctgtgactggcggaaggccctgttcctgttttccgcgtgacggtgctgaagcaggcgagatgcccctacactggagcccgctgcgtgactctgttaaccatgcctctggcgacgttctcattgatactaccacggcgccgctgagtcctgatggatgctcttttgcctttccgggtgttcgacacctgtcctcatcctgcgcggcatcgtcgtgttaacgcaggccatctgcggacaaggagacggcgtcgtgcaaacccattgaggaggcttcaacgtctggtcgcgacgcgacctccgcaagtcaccgtgcgggcccctcgtgtctaccggaggggccatcgtccctcaagccccgtcctgcgcgtgaagcgcggaatgctgcactcgttctctgcgcacctggtgtccaactgattatcacgcgaggttaaggaggaggtgatcaagccgacggaattcgactcctgcgacgtttttccaggagggtgcagacgacctgggacgcctgctggcagctcagctaccaccagccacggaggcgctgcgcgcctgagcctcaaggatgcgcacgagtacgactaccgcccctgatgtgtgtgcgaactggcgctcacctcctgtcgattgtcctctgcgcgctgtgctggctcgtgctgcgcgctggacatctgtcgtggatcgcgacccaacgacgcatcgtactgtgacgcgtccctgactcgtacacgtccgcctccagagtcacgtgcgcgctcaggtcagtcacggcctccgcctccagagtcacgtgcgcgcatgacgctgctcggacctgtgtgtgcggcggagcgaggagagcgcgaaaggctgttgccagaagaagcgcgcggttttgagatccccgctaccgcgactacgcggccctgtgcacggagggtcgcaggtgatgcacgccctggacggcgatcacggacctgagcaacgggacctgcgactgcacggagtctgtggagcgtatcacgcgccctgcccgctgagggcgctcggggagacgggtgtggaggcgcgcgacgcgtcgtcaactacattagcttttggcaccgccgctcggcgaggcgcggtggcaggcctgccgcacgggtgatggaccgacgcgctcgcgtgcctccatcctcgcagcgccttcacctggaggagggtgcgcgcggggcgccggaacggcaaggcgacggcctggagggtggcccccgcgagtcgtaccgctacgccgcgtgccctgtgtggagcaggggccgcaggcgaccgaggtcaactgcacgtcccgtcgtgctgtcgtcccatcgcacccagcgcattcccccaacagtttacgaagacgggtgctcagccctattctgaagaaggcgaccgcgtggtcgcgggcaactactggggaatcgccgtcggtggcgccctgcgaagtgtacgcgtcggtgattctcaaccgactcgtccgcgcggcgagacgctggacctccgcgacctgcgaagcgggcctccggctgttggaaccgcgcatcacctgttcgtgaagcagcacctgtgcggcgacgctcgtcggcggggtgcccgccctgcgtgtcggcgctgccccgcggTGAcacgcccgcgcggcgacctgacctgtctcgcaggggcgccctgtctgccttgcgaacaagccccctggagcatcggtgcatgctgtctgtgcgccccgcgtgcggtttgtgcctcgcggcgccgcggcgccggcgccgagcattgaattgttgcaaacccacctgacagattgagggcccaaggcaggaaggcgtgagatggaggctacatgaagttaactgaagttttatgataactaacaagaaggctgttctgcgcagcgcaatgacaagaacaagattcacattccgtgtagaggcttgccatcgaatgtgagcggcgccggccgcggacccgacaaaacccctacgacgtggtgaagaaaaacgtggcgggcactgtcctgtagcctgaagaccagcaggagacgatcggaagcatcacagcacaaccggtatcgatagcgtctgcgtgttgggagctggagtcgtggcgttgacgacggcgctgcagctcttcgaggatgtgcctggcgctgcgtcagtcgtgctgagaaatgtgcgacgaaacgttgacggcgtggggccgcggcgctgtggaatgcatacgatgggttacgcggccattggatgggattgtagtgcttatggaggataatagatgtttgtcggatccaacgcatgttgatgcagtgcggcgtgtgctgaaagtatggaaaggatgggtgattgtgctattcatcatgcgtgcgccacccttttcgcaggaaatgtgccggcaltgttggtgcaccgatggggaaatcagatttgcggcactacatgaagaattctgaaagatgcagcaactgggtgcgaaacggataacggttgttaggtgtatgtcgcagcatgtctggatttgcgggcaactccccctgcacggcccaattgttcaggttcgacgttgccttccgctgtgttctgtgtgaaattgt

[0242] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:VcLPAAT2 V2:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction the bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is

indicated by uppercase underlined text that enables amplification of the *VcLPAAT2* V2 gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *VcLPAAT2* V2. The initiator ATG and terminator TGA codons of the *CrLPAAT2* V2 are indicated in uppercase, bold italics, while the remainder of the gene is indicated in bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0243] Table 28 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK967. *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28°C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 28: Screen of primary transformants of CHK22 with pCHK967.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK967-1	0.05	1.62	21.57	3.09	58.33	12.72	0.61	0.28	0.11	1.55
pCHK967-2	0.05	1.61	24.49	2.75	59.06	9.64	0.56	0.26	0.09	1.45
pCHK967-3	0.05	1.64	22.82	3.13	58.83	11.12	0.56	0.31	0.10	1.53
pCHK967-4	0.05	1.59	22.40	3.26	58.98	11.26	0.57	0.30	0.10	1.57
pCHK967-5	0.06	1.68	22.50	3.31	58.05	11.91	0.60	0.30	0.10	1.57
CHK22 Control	0.04	1.56	26.48	3.06	58.93	7.59	0.47	0.27	0.07	1.56

Example 14: Expressing *Astrephomene gubernaculifera* LPAAT2 (*AgLPAAT2*) in *P. moriformis* strain CHK22.

[0244] *AgLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK968 contained 5' and 3' homology arms to permit targeted integration of *AgLPAAT2* into the genome and is shown in **Table 29**.

Table 29: Integrative sequences for the transformation of *P. moriformis* with pCHK968 encoding *AgLPAAT2*.

[illegible]

[illegible]

[0245] The construct can be written as

5'DAO1b::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmAMT03:Ag*LPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *AgLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *AgLPAAT2*. The initiator ATG and terminator TAG codons of the *AgLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0246] Table 30 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK968. The *P. moriformis* base strain CHK22 is shown as a non-transgenic

control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 30: Screen of primary transformants of CHK22 with pCHK968.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK968-1	0.06	1.67	20.64	3.35	57.91	13.90	0.64	0.32	0.11	1.59
pCHK968-2	0.07	2.09	19.46	2.71	51.79	21.09	0.82	0.23	0.17	2.21
pCHK968-3	0.05	1.59	21.25	3.49	58.21	12.97	0.59	0.30	0.11	1.83
pCHK968-4	0.05	1.53	20.67	3.25	58.74	13.26	0.57	0.31	0.11	1.53
pCHK968-5	0.05	1.52	21.18	3.25	58.34	13.19	0.60	0.30	0.11	1.63
pCHK968-6	0.05	1.54	24.14	3.28	58.66	9.94	0.53	0.30	0.08	1.64
CHK22 Control	0.04	1.56	26.48	3.06	58.93	7.59	0.47	0.27	0.07	1.56

Example 15: Expressing *Edaphochlamys debaryana* LPAAT2 (*EdLPAAT2*) in *P. moriformis* strain CHK22.

[0247] *EdLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK969 contained 5' and 3' homology arms to permit targeted integration of *EdLPAAT2* into the genome and is shown in **Table 31**.

Table 31: Integrative sequences for the transformation of *P. moriformis* with pCHK969 encoding *EdLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
27	gctcttcgcttgcgcgacccctcggtgacatcggagccctgcgcagcccttaaatcctcagtcagggtttctatgttcaactgagccctaaagggc tttcgtcacgcgcacgagcacacgtatcggctacgcagtcctcagaaagcggtagaaacagttcgcaagccctcggtcggtcgaaacttgcgc agtactatgtaattaaatgaatgatcgaatgagacgcgaacttttgcagaatgccactgagtttgcagagaaatgggagtgccgcattcacc atccgctgtgcacggcctgattcgcgcagacgatgaatggcgagaccaggagcggttgcgagcccgagccggtagcaggaaataacggtc gacaalcttctcgtcaattactggcaaccattagaaagagccggagcggttgaaattctgcaatcgagtaatttttcgaigcggcgggcctgct gaaccctaaagctccggcctatgtttaaggcgatccaagatgcacgcggcccgagccacgtgtctcaagcacaaccaccagccttagttcgaga ctttgggagatagcggcagatctatgtttggcattttgtatatttaattaaagtcgcgaatggagcgctctgatcggtgcagcgtcggtgcagca cctggcagttggcgctgggtcgccctatcgtcggcgctgggtgaagctggctcccgctctcgtcgggcgcgccCTTCTTGCGCTATGA CACTTCCAGCAAAAGGTAGGGCGGGCTGCCGAGACGGCTTCCCGGGCGCTGCATGCAACACCGA TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCTCTTCGTTTCAGTCACAACCCGCAAACTGTACATGctgctgagccct tctgttctcgtgctggcgttcgcgcgcaagatcagcgccctccatgaagaaagcagagcgtccgaccgccccctgtgcacttacccecaacaaggg ctggatgaacgaccccaacggcctgtggtacgacgagaaggacgccaagtggcacctgtacttccagtaaacccgaacgacacgctggtggga cgccctgttctggtggccacgcacgtccgacgacctgaacaaactgggaggaacagcccatcgccatcgccccgaagcgcaacgactcggcgcc ttccggctccatggtgtggtgactacaacaacacctccggcttcttcacacgacaccatcgaccgcgcagcgctggtggccatctggacctaca caacccgagtcgagggagcaglacatctctacagcctggacggcggtacacctcaccgagtaaccagaagaaccccggtggtgcccgaact ccaccaggttcgcgacccgaagggtctctgtgacgagccctccagaagtggatcatgaccgcggccaagtccaggaactacaagatcgagatct actctcgcgacgacctgaagtcctggaagctgagtcgggttcgcgaacgagggtctcctcggtacagtaagagtgcccgccctgacgaggt cccaccgagcaggacccagcaagtcctactgggtgagttcctcctccatcaaccccgggcgccccggcggtccttcaaccagttacttctg

[illegible]

SEQ ID NO:	Nucleotide Sequence
	tggtgactggaggatacgaactttcgccgtcaaatteccagaggaggacccctctgggcccacattgtgcccaactgctcttccgcttgcgtgttcc tgtgtgaaattgt

[0248] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*EdLPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *EdLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *EdLPAAT2*. The initiator ATG and terminator TAG codons of the *EdLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0249] Table 32 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK969. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 32: Screen of primary transformants of CHK22 with pCHK969.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK969-1	0.05	1.58	21.56	3.58	57.99	12.96	0.58	0.25	0.12	3.36
pCHK969-2	0.05	1.60	21.25	3.51	57.37	13.94	0.56	0.22	0.12	3.78
pCHK969-3	0.05	1.57	20.99	3.28	58.55	13.11	0.60	0.32	0.11	1.53
pCHK969-4	0.05	1.51	22.39	3.25	59.01	11.41	0.56	0.31	0.10	1.55
pCHK969-5	0.04	1.52	24.72	3.10	59.05	9.28	0.50	0.29	0.09	1.54
pCHK969-6	0.05	1.68	26.00	3.09	57.68	9.20	0.51	0.29	0.09	2.19
CHK22 Control	0.04	1.56	26.48	3.06	58.93	7.59	0.47	0.27	0.07	1.56

Example 16: Expressing *Dunaliella salina* LPAAT2 (DsLPAAT2) in *P. moriformis* strain CHK22.

[0250] *DsLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK971 contained 5' and 3' homology arms to permit targeted integration of *DsLPAAT2* into the genome and is shown in **Table 33**.

Table 33: Integrative sequences for the transformation of *P. moriformis* with pCHK971 encoding *DsLPAAT2*.

[illegible]

SEQ ID NO:	Nucleotide Sequence
	TCAGTTGAAGGGCTTTACGCGCAAGGTACAGCCGCTCCTGCAAGGCTGCGTGGTGGAAATTGGACG
	TGCAGGTCTCTGCTGAAGTTCTCTCCACCGCTCAACAGCGGACAAAGCACCGGTGTATCAGGTCCGT
	GTCATCCACTCTAAAGAGCTCGACTACGACCTACTGATGGCCCTAGATTCTTCATCAAAAAACGCTG
	AGACACTTGCCAGGATTGAAACTCCCTGAAGGGACCACCAGGGGCCCTGAGTTGTTCTTCCCCC
	CGTGGCGAGCTGCCAGCCAGGCTGTACCTGTGATCGAGGCTGGCGGGAAAAATAGGCTTCGTGTGC
	TCAGGTCATGGGAGGTGCAGGACAGCTCATGAAACGCCAACAAATCGCACAAATTCATGTCAAGCTAA
	TCAGCTATTTCCTCTTACAGAGCTGTAATTGTCCCAAAATTCGTGCTACCGGGGGTGATCCTTCGT
	GTACGGGGCCCTTCCCTCAACCCCTAGGTATGCGCGCATGCGGTGCGCCGCGCAACTCGCGCGAGGG
	CCGAGGGTTTGGGACGGGGCCGTCCCGAAATGCAGTTGCACCCGGATGCGTGGCACCTTTTTTGCG
	ATAATTTATGCAATGGACTGCTCTGCAAAATTCGGCTCTGTGCGCAACCCCTAGGATCAGCGGCGTA
	GOATTTCTGAATCATTCTGCTCTGATGGGAGCTACCGACTACCCCTAATATCAGCCCCGACTGCTGAC
	GCCAGCGTCCACTTTTGTGCACACATTCCATTCTGTGCCCAAGACATTTTCATTGTGGTGGGAAGCGTC
	CCCAGTTACGCTCACTGTTTCCCGACCTCCTTACTGTTCTGTGCGACAGAGCGGGCCCCACAGGCCG
	GTGCGAGCCGGTACATGggggagcctctgctcagaaghtcctctcctgctcactctctctggggahtctctctgtactgtgctctc
	cgahtcgtgctgctggctggcauccctgaagctctctggathtcactctcctggagcagatgtacactgttggttcagttatgaccnaagctgttccgat
	caagatcaagaagtactggccctaacggcctgctgcgaaggacacgggcggccacatctctactctgtgcaaccaccgctctgtggacgcagcttttctg
	gacgcttactgtacggaggggcaacggctcgtctatgtcgcctgtgctgtgttctcggctctcccggtctttatgtgtggcggtgactgtgctgcgggag
	tcactctgttcaagcggggcacgggtctgcacaaggagaaghtcaacgagtggtggtactcgaaggtgtctctctcgcgactccggcactgtctgtct
	accccgaggggcaccgctctcctcgtctatgaagagctcccgctcgaagcggggatgtgtactacgcgcactccgaagtttccgtgcagatcatt
	atcagcgcaaacaggaggaggtgtgtctgcagaagacgatgaaggcccaattgttggtgcacgactgtcactgggtctctcgtgactgactgagtc
	ggggcgaagacatcaggaggtctctgagcgaggctcagacggctctgggcaagaggtctgtgactctgtgtactcgcggacgtctgcgcctccc
	cgagctggagcatucggacgtctcccaagttcgaactctgtttgcatgtgcgcgtgactgagctgtgtctcggcaccgltgtgtctctcggc
	acgatgtgcggcactgttcaagttgtgtgtgtgtgtctcgcgcactcggggcgacctctggctgcgaaggtgtctgtcagctcgtctgtgtgtgtgtct
	gtgtctactctgaacagccgagcgccctctcggcaagTGAacacggccgcgcggcgccacctgacctgtgtctctcagaggccgctgtgtctcccttgcgaa
	acaagccctctggagcatgcgtgcatgatgtctctgtgcgcccccgcgcgcggtttgtgcctctgcggcgccggcgccggcgccggcgccgcatgaaatt
	gttgcgaaccccaactgacagattgaggcgccagcaggaagcgctttagatgagggtacagggatcgaatgaatgaatgaatgtttatgataactaacaca
	aaaggctctgttctgcccagcgaatgacgaagaacaagattccacatttccgttagagcgttgcagcttcgcatcgaatctgagccggcgccgcgaccgcaca
	aaaccccttaccgacgtggttaagaaanaacgtgtgcggcactgtctccctgttagcctgaagaccagcagcgaagcagtcggaagcatcaccagcacaaccggtat
	cgatagcgctctgctgtgttggagctggagctgtgtggcctgacgacggcgctgcagcctctcaggaatgtgctgtgagctgtgcggttcaagctgtgtgc
	tgagaaatattggcgacgaaacgttgacggctggggcgccggcggtgtggatgccatagcattgggtacggcgccattggatgggattggtagg
	cttatggaggatantagagtttttctgggacccaacgcatgtgtgatgcagtagcccggtgtgtgtgctgaagatgaggaaggtgtgtgcatgtgtatc
	acatgtgctgcccaaccttttgcaggaaatgtgcggcactgtgtgtgacccgatgggggaaatcgacgttgcaccactacatgaaatgat
	accttgaagatgcagcaacgtcgggtgtgcgaacggataaacggtttggtaggtgtatgtcgcagcagatgtgtgatttttggggcaactccctgt
	ccacggccattgcagggtgtcatgtgtgactgtgaggtatgcaacttgcggcgtcaaaattccagaggaggaggaacccctctgtggcgcgacattgtgcgc
	actgtctctcgtctgtgttctctgtgaaattgt

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *DsLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text,

drives the expression of the *DsLPAAT2*. The initiator ATG and terminator TGA codons of the *DsLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0252] Table 34 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK971. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 34: Screen of primary transformants of CHK22 with pCHK971.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK971-1	0.06	1.56	21.15	3.35	56.64	14.85	0.63	0.23	0.11	2.16
pCHK971-2	0.06	1.58	21.67	3.56	56.73	14.04	0.60	0.22	0.08	2.35
pCHK971-3	0.06	1.58	20.50	3.36	56.76	15.24	0.67	0.24	0.08	2.11
pCHK971-4	0.05	1.51	20.65	3.44	57.15	14.86	0.57	0.24	0.10	2.20
pCHK971-5	0.04	1.44	24.31	3.15	59.43	9.30	0.49	0.30	0.09	1.57
pCHK971-6	0.06	1.52	19.88	3.23	56.45	16.58	0.60	0.23	0.11	2.24
CHK22 Control	0.04	1.56	26.48	3.06	58.93	7.59	0.47	0.27	0.07	1.56

Example 17: Expressing *Scenedesmus* sp. *LPAAT2* (*SceLPAAT2*) in *P. moriformis* strain CHK22.

[0253] *SceLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK973 contained 5' and 3' homology arms to permit targeted integration of *SceLPAAT2* into the genome and is shown in Table 35.

Table 35: Integrative sequences for the transformation of *P. moriformis* with pCHK973 encoding *SceLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
29	gctcttcegettgccegcacctcggtgatctgggagcctgcgcagcccttaaatcatctcagtcagggttttatgttcaactgagcctaaagggc tttcgtcacgegcacgagcacacgtatateggtacgcagtcctcagaaagggtagaaacagtttcgcaagccctcgtcggtcgaaaacttgcgc agtactattgaattaaattaantgatcgatgagacgcgaaacttttcagaaatgcaactgagtttgcagagaaatgggagtggeccattcacc atccgctgtgcacggcctgattcccgagacgatgaatggcgagaccaggagcggttgcagagcccgagcggtagcagggaataacggte

Nucleotide Sequence

[illegible]

[0254] The construct can be written as

5'DAO1b::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmAMT03:Sc*LPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *SceLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *SceLPAAT2*. The initiator ATG and terminator TGA codons of the *SceLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0255] Table 36 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK973. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct

transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 36: Screen of primary transformants of CHK22 with pCHK973.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK973-1	0.05	1.54	23.14	3.14	58.67	11.06	0.57	0.30	0.10	1.56
pCHK973-2	0.06	1.51	21.14	3.51	55.80	15.58	0.63	0.24	0.08	1.64
pCHK973-3	0.05	1.51	22.45	3.02	58.88	11.65	0.52	0.28	0.10	1.62
pCHK973-4	0.07	1.65	19.94	3.49	54.37	17.96	0.68	0.26	0.12	1.59
pCHK973-5	0.06	1.65	20.99	3.57	54.54	16.80	0.63	0.24	0.12	1.54
pCHK973-6	0.06	1.56	21.96	3.40	55.61	15.04	0.59	0.24	0.11	1.64
CHK22 Control	0.04	1.56	26.48	3.06	58.93	7.59	0.47	0.27	0.07	1.56

Example 18: Expressing *Micractinum conductrix* LPAAT2 (McLPAAT2) in *P. moriformis* strain CHK22.

[0256] *McLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK974 contained 5' and 3' homology arms to permit targeted integration of *McLPAAT2* into the genome and is shown in **Table 37**.

Table 37: Integrative sequences for the transformation of *P. moriformis* with pCHK974 encoding *McLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
30	gctcttccgcttgcgccacccctcggtgatctgggagccctgcgcagcccttaaaatcatctcagtcagggtttctatgtttcaactgagcctaaggggc tttcgtcacgcgcacgagcacacgtatattcggtacgcagctctctcagaagcggtagaacagtttcgcaagccctcgtcggctcgaanaacttgcgc agtaactatgaattaaattaaatgacgaatgagacgcggaacttttgcagaatgccacttgaatttgcagagaaatgggagttggcgcaattcacc atccgcctgttgcacggcctagatttcgcgagacgaatggcgagaccaggagcggcttgcgcagcccgcggttagcgaataacgggtc gacaatcttctgttccaaattactgggcaaccattaganaagagccggagcggcttgaanaattctgcaatcgagtaatttttcgatgcggcgccgtct gaaaccttaaggetcggcctatgttttaaggcgatccaagatgcacgcgggccccaggaacagttgtctcaugcaacaaacccagccttagtttcgaga ctttgggagatagcggcagatattctagtttggcattttgtatattaaatgaatctcgaatggagcgtctgtatgcgttgcagcgttcggctgcagca ccctggcagtgccgtctggggtgcgccctatcgtctggcgccctggctacgttgcgtctccgcctcctgaggcgccgcctCTTTCTTTCGCGCTATGTA CACCTTCCAGCAAAAGGTAAGGCGCGGCTGCGAGACGGCTTCCCGCGCTGCATGCAACACCGA TGATGCTTTCAGACCCCCGCAAGCTCCTTCGGGGCTGCATGGCGGCTCCGATGCCGTCCAGGOC GAGCGCTGTTTAAATAGCCAGGCCCCCCGATTGCAAAAGACATTTATACGAGCTTACCAAAAGCCA TATTCAAACACCTTACATCACTACCACTTCTACACAGGCCACTTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCCCTCTTCGTTTCAGTCACAACCCCGCAAACTGTACATGctgtcagggcct tcctgttctgtgtgcggggttcgcgcgaagatcagcgcctccatgacgaacagagacgtccgaacgccctcgtgtgcacttcccccaacaagg ctggatgaacgaccccaacggcctgtgtgtacgacgagaaggacgcccaatgggacccctgttcttcagtaaacccgaacgacacgtctggggg cgcctgtgtgtggggccacgccacgtccgacgacctggaacacgtggaggaacccatcgccatcgcccggaagcgcacgactcgggcgc ttctcgggtccatgtgtgtgactacaacaacacctcggccttcttcacgacaccatcgacccgcgcagcgtgtgtgccatctggaactacaa caccccgagctcagagagcagatagctctctacacgtctcgaacgcgcctacaccttaccgagttaccagaaagacccctctcgtcgcgcgaact

[illegible]

SEQ ID NO:	Nucleotide Sequence
	aaaccctacgacgtggttaagaaaaacgtggcggcgactgtccctgtagccgtgaagaccagcaggagacgacgacgaaagcattccacgcacaaccggat cgataggcgtctgcgtgttgggagctggagctcgtggccttgacgacggcgctgcagcctctgcaggatgtgccctggcgctgcgttcaacgtctggac tgagaaatatggcgacgaaacgttgacggctggggcgccggcgctgtggatgccatacgcattgggtacgcggccattggatgggattggtagg cttatggagggtataatagatttttctggatccaacgcattgtatgcagtagcccggtgtctgtaaaagtatggaaggatggtgcatgtggtattc acatggcgtgcccaacccttttcgaggaaatgtgccggcatcgttgggtgcacgatggggaaaaatcgacgttcgaccatacatgaaaatgtat acctctgaagatgcagcaactcggggtgcgaaacggataaacggtttggtaggtatgtgcagcatgtctggaattttcgggcgaactccccctg ccacggcccatgcagggtgtcattgttgactggaggatagcaactttcggcgctcaaaatccagaggaggaccctctgggcggacattgtgcc actgtctctccgttctgttctgtgtgnaattgt

[0257] The construct can be written as

5'DAO1b::CrTUB2:*ScSUC2*:PmPGH:CvNR:PmAMT03:*McLPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *McLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *McLPAAT2*. The initiator ATG and terminator TGA codons of the *McLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0258] Table 38 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK974. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 38: Screen of primary transformants of CHK22 with pCHK974.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK974-1	0.04	1.51	23.69	3.15	59.18	10.11	0.51	0.32	0.10	1.54

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK974-2	0.06	1.68	21.27	3.61	57.79	13.10	0.59	0.33	0.11	1.49
pCHK974-3	0.05	1.58	22.76	3.41	58.49	11.19	0.55	0.31	0.07	1.52
pCHK974-4	0.05	1.51	20.53	3.45	58.36	13.52	0.58	0.32	0.11	1.56
pCHK974-5	0.05	1.60	22.41	3.12	58.05	12.12	0.54	0.28	0.10	1.55
pCHK974-6	0.04	1.49	24.72	3.15	59.16	9.06	0.51	0.28	0.07	1.54
CHK22 Control	0.04	1.56	26.48	3.06	58.93	7.59	0.47	0.27	0.07	1.56

Example 19: Expressing *Chlorella sorokiniana* LPAAT2 (*ChsoLPAAT2*) in *P. moriformis* strain CHK22.

[0259] *ChsoLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK975 contained 5' and 3' homology arms to permit targeted integration of *ChsoLPAAT2* into the genome and is shown in **Table 39**.

Table 39: Integrative sequences for the transformation of *P. moriformis* with pCHK975 encoding *ChsoLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
31	gctcttcgcgttgcgcgcacccctcggtgatctgggagccctgcgcagcccttaaatcatctcagtcagggtttctatgttcaactgagcctaagaaggc tttctgcacgcgcacgagcacacgtatatacggctacgcagctctctcagaagccggtagaaacagtttgcgaagccctctgcgtcgaanaacttgcgc agtactattgaanfaaataaataatgatcgaatgagacgcgaacttttgcagaatgccactagtttgcgcagagaatgggagttggcgccattcacc alcgcctctgtgcagcgctgattcgcgcgagacgatgaatggcgagacaggagagcggttgcgcagcccgtagccggaataaacgggtc gacaattctctctgccaactactggcaaccattagaaaagagccggagcgcggttgaanaattctgcaatcagagtaaattttctgatcgccggcgccctgtc gaaacctaaaggctccggcctatgttttaaggcgatcacaagatgcacgcggcccgaggcacgtgtctcaagcacaaacccagccttagtttgcaga cttggggagatagcggcagatatactagtttggcattttgtatataaataaagctcgcgaatggagcgctctgatgcggtgcagcgctcgtctgcaga cctggcagttggcgctggggtcgccctatcgtctggcgccctgggtcagctggctccgcctctctgcggcgccctCTTTCTTGCGCTATGA CACTTCCAGCAAAAGGTAGGGCCGGGCTGCGAGACGGCTTCCCGCGGCTGCGATGCAACACCGGA TGATGCTTTCGACCCCCCCGAAGCTCCTTCGGGGCTGCATGGCGGCTCCGATGCCGCTCCAGGGC GAGCGTGCTTTTAAATAGCCAGGCCCCCCGATTGCAAAAGACATTTATAGCGAGCTACCCAAGGCCA TATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCTCTTCGTTTCAGTCACAACCCGCAAACTGTACATGCTgctgcaggcct tctgttctctgtcgtgcgggttgcgcgaagatcagcgcttccatgacgaacgagacgctcgaacgcgccctctgtgtacttaccaccaacaaggg ctggatgaacgacccaacggcctgtgtgtacgacgagaaggaacgccaagtggcacctgttacttccagtaaacccgaacgacacccgtctggggga cgccctgttcttggggcgaacgcacgtccgacgacctgaacaaactgggaggaacagcccatcgcctcgcgcgcgaagcgaacgactcggcgcc ttctcggctcatgtgtgtggtgactacaacaacacctccggcttcttaacgaacacatcgaacccgcgcagcgctgcgtggccatctggacttaaa caaccgcggagtcgaggagcagtaacttctacagccttggacggcggtctacacttaccgagtaaccagaagaaccccgtgtgtgcgcgaact ccaccagttcgcgcgaagctgttctgtgtacgcctccagaaggtgactgacacgcgcgaagtcgcgcgaagtaacgaagactgaagatct actctccgacgactgaagtctgtggaagctgggtgtccgcgttcgcaacggagggcttctcgtctccagtaacgagtgcccgccctgctcaggt ccccaacgagcagggaacccagaagtcctactgtgtgtatgttacttctcatcaaacccggcgcccgccggcggtccttcaaccagttacttgc gcagcttcaacggcaccacacttgcggccttcgacaacacagttccgcgtgtgtgacttgcgaaggaactactacgacctgacagacttcttaacac gaccgcacttaccgaagcgcccttggcactgcgtgtggccctccaacttggagtaactcgccttctgtgcccaacccccctggcgctctccatgctct ctgtgcgaagttcttctcaaacacgagtaaccggaaccccggaagcggagctgatacaactgaaggcgccgagatcctgaacatcagcaacg cgcgcctctgaccccgcttcccaacacagccactgttaccgaacgcgaacactgaacacgtctcttccagcagcccgcccttgcgaagttc

[0260] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*ChsoLPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *ChsoLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *Chlorella vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *ChsoLPAAT2*. The initiator ATG and terminator TGA codons of the *ChsoLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0261] **Table 40** shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK975. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 40: Screen of primary transformants of CHK22 with pCHK975.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK975-1	0.07	0.03	20.71	3.44	57.62	14.19	0.59	0.26	0.12	1.53
pCHK975-2	0.05	0.02	24.43	3.08	59.18	9.49	0.51	0.24	0.08	1.51
pCHK975-3	0.06	0.02	20.71	3.4	57.82	14.16	0.57	0.24	0.11	1.49
pCHK975-4	0.06	0.02	20.95	3.46	57.31	14.28	0.59	0.26	0.12	1.50
pCHK975-5	0.06	0.02	20.92	3.5	57.12	14.39	0.57	0.25	0.11	1.51
pCHK975-6	0.07	0.03	20.86	3.34	56.95	14.76	0.59	0.23	0.1	1.45
CHK22 Control	0.04	0.02	26.18	2.95	59.62	7.55	0.46	0.24	0.07	1.49

Example 20: Expressing *Chlorella variabilis* LPAAT2 (*ChvaLPAAT2*) in *P. moriformis* strain CHK22.

[illegible]

[0263] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:ChvaLPAAT2:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *ChvaLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *ChvaLPAAT2*. The initiator ATG and terminator TAG codons of the

ChvaLPAAT2 are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0264] Table 42 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK976. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 42: Screen of primary transformants of CHK22 with pCHK976.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK976-1	0.05	0.02	25.29	3.1	60.41	7.57	0.45	0.26	0.08	1.56
pCHK976-2	0.05	0.02	23.82	3.11	59.49	9.74	0.5	0.27	0.09	1.51
pCHK976-3	0.05	0.02	23.74	3.03	60.23	9.12	0.48	0.28	0.09	1.51
pCHK976-4	0.05	0.02	23.45	3.19	59.56	9.93	0.54	0.3	0.1	1.58
pCHK976-5	0.04	0.02	24.7	3.06	60.13	8.36	0.51	0.26	0.08	1.56
pCHK976-6	0.06	0.03	21.49	3.27	58.89	12.13	0.57	0.3	0.11	1.50
CHK22 Control	0.04	0.02	26.18	2.95	59.62	7.55	0.46	0.24	0.07	1.49

Example 21: Expressing *Raphidocelis subcapitata* LPAAT2 (*RsLPAAT2*) in *P. moriformis* strain CHK22.

[0265] *RsLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK977 contained 5' and 3' homology arms to permit targeted integration of *RsLPAAT2* into the genome and is shown in **Table 43**.

Table 44: Integrative sequences for the transformation of *P. moriformis* with pCHK977 encoding *RsLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
33	gctcttcgcttgcgcgaacctcggtgatctgggagccctgcgcagccctttaaatacatctcagtcaggcttctatgttcaactgagccctaaggcc ttctgtcacgcgcagcagcacacgtatctcggtacgcagctctcagaagcggtagaacagttcgcaagccctcgtcggcgaacttgcgc agtactatigaattaaattaatgatcgatgagacgcgaaactttgcagaatgccactgagtttgcagagaaatgggagtgccgcattcacc atcgcctgtgcacggccgatctgcgcgagacgatgaatggcgagaccaggagcggcttgcagagcccgagccggtagcagggaataacggc gacaatcttctcgaattacitggcaaccattagaaagagccggagcggcttgaattctgcaatcgagtaattttcagatgcggcggccctgct

[illegible]

[illegible]

[0266] The construct can be written as

5'DAO1b::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmAMT03:Rs*LPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *RsLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *RsLPAAT2*. The initiator ATG and terminator TAG codons of the *RsLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0267] Table 44 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK977. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct

transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 44: Screen of primary transformants of CHK22 with pCHK977.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK977-1	0.06	0.04	21.34	3.17	56.63	14.69	0.57	0.24	0.1	1.46
pCHK977-2	0.06	0.02	20.32	3.34	55.75	16.31	0.58	0.25	0.11	1.53
pCHK977-3	0.06	0.04	19.94	3.22	56	16.65	0.6	0.24	0.12	1.48
pCHK977-4	0.06	0.03	21.97	3.25	56.97	13.74	0.54	0.24	0.1	1.46
pCHK977-5	0.05	0.02	25.83	2.99	59.44	7.9	0.46	0.25	0.08	1.58
pCHK977-6	0.04	0.02	26.09	3.12	59.68	7.43	0.44	0.27	0.08	1.55
CHK22 Control	0.04	0.02	26.18	2.95	59.62	7.55	0.46	0.24	0.07	1.49

Example 22: Expressing *Chlorella desiccata* LPAAT2 (*ChdeLPAAT2*) in *P. moriformis* strain CHK22.

[0268] *ChdeLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK978 contained 5' and 3' homology arms to permit targeted integration of *ChdeLPAAT2* into the genome and is shown in **Table 45**.

Table 45: Integrative sequences for the transformation of *P. moriformis* with pCHK978 encoding *ChdeLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
34	gctcttcctgctgcccgcacctcgttgatctgggagccctgcgcagcccttaaatcatctcagtcaggttctatgttcaactgagcctaaggge ittcgtcacgcgcagcagcagcgtatctcgtctacgcagctctctcagaaagggtagaaacagttcgcnaagccctcgtcggcgaanaacttgcgc agtactatfgaattaaatgaatgatcgaatgagacgcgaacttttgcagaatgccacigagtttgcgcagagaatgggagtggegcattcacc atccgctgtgcaaggccctgattcgcgcagacgatgaatggcgagaccaggagcggcttgcgcagcccgagccggtagcaggaataacgctc gacaaatcttctgtccaattactggcaaccattagaaagagccggagcgcgttgaaattctgcaatcagtaattttcgtatggcgccggcctgct gaaccctaaggctccggcctatgtttaaggcgatccaagatgcacgcggcccccagggaegtgtctcaagcacaacccagccctagtttgcaga ctttgggagatagcggcagatattctagtttggcattttgtatattaattaaagtcctcgaatggagcgtctgtatgcggtgcagcgtcggctgcagca cctggcagtgccgctggggtcgcctatcgtctggcgccctggctcagctggtctccgcctccctggcgccgcctCTTTCTTGGCGCTATGA CACTTCCAGCAAAAGGTAAGGCGCGGCTGCGAGACGGCTTCCCGGCGCTGCATGCAACACCGA TGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGOC GAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACCTTCTACACAGGCCACTCGAGCTTGTGATCGCACTCCG CTAAGGGGGCGCCTCTTCTCTTTCGTTTCAGTACACAACCCGCAAACTGTACATATGctgtgcaggcct tctgttctgtggtgcccgttcgtccgccaagatcagcgcctccatgacgaacgagacgtccgaccgcccttgggtgcactcacccecaacaagg ctggtatgaacgaccccaacggcctgtgtgacgacgagaaggacgccaaagtggcactgtacttccagtaacccgaacgacacccgtctggggga cgccttgttctggggccacgcacgtccgacgacctgacaaactggaggaaccagcccatcgcacccgcccggaagcgaacgactccggcgc ttctccggtccatgtgtgtgactacaacaacacccctccggtcttcaacgacacccatcgaaccgcgcagcgtcgtgtgacctgtggaactacaa caccceggagtcggagagcagtaactctacagcctggagcgcggtacacctcaccgagtaaccagaagaaccccgctgtgcccgcacact

[illegible]

SEQ ID NO:	Nucleotide Sequence
	caggatgtccctggcggtgcggttcacgtcgtggtgagaatatggcgacgaaacgttgacggctggggccggcggtgtggtatgccatacgcattgggtacgcggccattggatgggattgtaggcttatggaggataatagagttttgtcggatccaacgcatgttgatgcagtagcccggtgtgctgaaagiatgggaaggaatgggtcattggctattcacatgcgtgccaccccttttcaggaaaatgtgcggcatcgttggtgcaccgatggggaaaatcgacgttcgaccactacatgaaaatgtataacctgaagatgcagcaactgcgggtgcgaaacggataacggtttggtagggtatgtcgagcatgtgctggattttgcgggcaactcccctgccacggccattgcagggtgcaigtgtgactggaggatacgaacttcggccgctcaaatccagaggaggacccctcctgggcccgaattgtgccacigtctcttcggttgcgttttcctgtgtgaaattgt

[0269] The construct can be written as 5’DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:ChdeLPAAT2:PmPGH::3’DAO1b. Proceeding in the 5’ to 3’ direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β-tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *ChdeLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *ChdeLPAAT2*. The initiator ATG and terminator TAG codons of the *ChdeLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0270] Table 46 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK978. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 46: Screen of primary transformants of CHK22 with pCHK978.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK978-1	0.04	0.02	25.77	3.1	60.01	7.39	0.48	0.26	0.08	1.72
pCHK978-2	0.05	0.04	24.4	3.07	56.47	11.66	0.52	0.34	0.1	1.70
pCHK978-3	0.06	0.03	21.29	3.2	59.24	12.16	0.55	0.29	0.1	1.55

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK978-4	0.06	0.03	21.03	3.39	59.09	12.34	0.55	0.31	0.11	1.56
pCHK978-5	0.05	0.02	22.31	3.05	59.53	11.05	0.53	0.28	0.1	1.56
pCHK978-6	0.05	0.03	21.5	3.18	59.22	11.9	0.56	0.29	0.11	1.67
CHK22 Control	0.04	0.02	26.18	2.95	59.62	7.55	0.46	0.24	0.07	1.49

Example 23: Expressing *Auxenochlorella proteothecoides* LPAAT2 (ApLPAAT2) in *P. moriformis* strain CHK22.

[0271] *ApLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK979 contained 5' and 3' homology arms to permit targeted integration of *ApLPAAT2* into the genome and is shown in **Table 47**.

Table 47: Integrative sequences for the transformation of *P. moriformis* with pCHK979 encoding *ApLPAAT2*.

[illegible]

[illegible]

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*ApLPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *ApLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *ApLPAAT2*. The initiator ATG and terminator TAG codons of the *ApLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0273] Table 48 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK979. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 48: Screen of primary transformants of CHK22 with pCHK979.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK979-1	0.04	0.03	25.91	3.07	60.15	7.22	0.46	0.26	0.08	1.47
pCHK979-2	0.06	0.04	21.27	3.41	57.2	14.02	0.54	0.26	0.11	1.58
pCHK979-3	0.05	0.05	21.35	3.6	58.07	12.97	0.52	0.28	0.11	1.58
pCHK979-4	0.06	0.03	21.43	3.39	58.06	13.06	0.54	0.27	0.1	1.55
pCHK979-5	0.07	0.04	20.44	3.54	56.79	15.01	0.58	0.27	0.11	1.56
pCHK979-6	0.05	0.03	24.9	3.03	59.02	9.16	0.48	0.25	0.08	1.54
CHK22 Control	0.04	0.02	26.18	2.95	59.62	7.55	0.46	0.24	0.07	1.49

Example 24: Expressing *Chloropicon primus* LPAAT2 (*ChprLPAAT2*) in *P. moriformis* strain CHK22.

[illegible]

[0275] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*ChprLPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *ChprLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *ChprLPAAT2*. The initiator ATG and terminator TAG codons of the *ChprLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is

indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0276] **Table 50** shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK980. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 50: Screen of primary transformants of CHK22 with pCHK980.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK980-1	0.04	0.02	21.31	2.97	61.56	10.33	0.64	0.27	0.1	1.44
pCHK980-2	0.06	0.03	21.32	3.43	58.83	12.25	0.56	0.3	0.11	1.48
pCHK980-3	0.06	0.03	21.18	3.42	58.51	12.63	0.53	0.3	0.11	1.51
pCHK980-4	0.06	0.03	22.41	3.1	59.03	11.24	0.53	0.28	0.1	1.54
pCHK980-5	0.06	0.03	21.29	3.32	58.8	12.37	0.55	0.3	0.11	1.43
pCHK980-6	0.05	0.03	20.8	3.37	58.58	13.04	0.57	0.31	0.11	1.62
CHK22 Control	0.04	0.02	26.18	2.95	59.62	7.55	0.46	0.24	0.07	1.49

Example 25: Expressing *Homo sapiens* AGPAT1 in *P. moriformis* strain CHK22.

[0277] *AGPAT1* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK966 contained 5' and 3' homology arms to permit targeted integration of *AGPAT1* into the genome and is shown in **Table 51**.

Table 51: Integrative sequences for the transformation of *P. moriformis* with pCHK966 encoding *AGPAT1*.

SEQ ID NO:	Nucleotide Sequence
37	gctcttcgcttgccegcacccctcgttgatcgtggagccctgcgcagcccccataatcatctcagtcagggtttctatgttcaactgagccctaaaggcc tttcgtcagcgcagcagcagcacagctatcggctacgcagctctcagaagcggtagaacagttcgaagccctcgtcggcgaanaacttcgcc agtactattgaattaaattaatgatcgaatgagaecgcgaacttttcgagaatgcactgagtttcccagagaatggagttggcgccattcacc atccgctgtgcagcgcctgatttcgcgagacgatgaatggcgagaccaggggagcggcttgcgagcccccagcgggtagcaggaataaacggtc gacaatctctcgtccaaacttggaacattagaaagagcggagcggcttgaaattctgcaatcgagtaattttcgaatgcggcgccctgct gaaccctaaggctccgacctatgtttaaggcgatccaagatgcacgcggcccccaggcaggtgtctcaagcacaaccccagccttagttcgaga ctttgggagatagcggcagatatctagtttggcattttgtatataaataaagctcgcgaatggagcgcctgagtcgggtgcagcgtcggctgcagca ccctggcagtgccgtggggtgcctctatcgtcggcgccctggctcagctggctcccgccctcctgcggcgccgccttcttcttgcgctatga CACITCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGGCGCTGCATGCAACACCGA

[illegible]

SEQ ID NO:	Nucleotide Sequence
	<u>atgtcgcacgtgttcgggagatcagcaccgatggccgcgggaggcgactacctgaagaagccggcgggcggtgTA Gacgcgccgcg</u> <u>gcgaactgaactgttcctcga gggcgccgtgtctgcttgc gaaacaaagcccccgagcatgcgtgc atgacgtctctgcgc ccccgccgcgcgtti</u> <u>gtgcgccctgcgcggcgccacggccgcggggcgacattga aattgttgc aaaccccaacctgacagattgagccccaggcgaggaa ggcgttgatgg</u> <u>aggtacagagggtcagaaacgtgaa gttttatgataactaacacaa aaggtcgtttctgtccacgcgaatgacaa gaaacaggttccacattccgtgtga</u> <u>ggcttgccatcgaatgtggcgggcg gggcccgcgaccgcgcacaaaccccttaccgacgttggtta gaaanaacgtgcgcgcactgtcccgtagcccgaa</u> <u>accagcgagacacgtatgcgcacatcagacacacacgggtatcgatagcgttcggtgtggagcgtgagtcgtgtggcgttgacgacggcgctgc</u> <u>agctcttgcaggatgtgcccggcggtgcgcgttcaacgtcggtcgagaaatattggcgacgaaacgtttgacggcgtggggcgggcggtgcgtggatg</u> <u>ccatagccattgggtacgcggccattggaatgggtatggtaggcttatggaggggataatagagttttgtcggaatccaacgcattgttgatcagtag</u> <u>cccgggtgtgctgaaagtatggaa ggaatggtgcatttggctattcacatgcgcgccaccccttttcgcaggaaatgtgcgggcacgttggtgcac</u> <u>cgatggggaa aatcgacgtttcgaccacatcatgaaaatgtatacctctgaagatcagcaacactcggggtcgaaacggalaacggtttggtagg</u> <u>tgtatgtcgagcatgtgtcgtga ttttgcgggcaactccccctgcacaggccacttgcagggtgcatgttgactggaggatagcaacgttccggcgt</u> <u>caaatcccagaggaggaa cccctctggggcgacattgtgccactgctcttcgcgttgcgtgttccgtgtgtaaatgtt</u>

[0278] The construct can be written as

5'DAO1b::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmAMT03:*AGPAT1*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *AGPAT1* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *AGPAT1*. The initiator ATG and terminator TAG codons of the *AGPAT1* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0279] Table 52 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK966. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 52: Screen of primary transformants of CHK22 with pCHK966.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	

pCHK966-1	0.05	1.54	20.69	3.16	59.29	12.79	0.58	0.32	0.00	1.58
pCHK966-2	0.05	1.51	20.60	3.66	57.62	14.29	0.54	0.23	0.12	3.6
pCHK966-3	0.11	2.16	20.07	2.85	53.51	18.23	0.78	0.14	0.00	3.49
pCHK966-4	0.04	1.50	23.85	3.07	59.39	9.80	0.52	0.31	0.09	1.59
pCHK966-5	0.06	1.60	20.73	3.14	58.41	13.59	0.60	0.30	0.11	1.53
pCHK966-6	0.06	1.66	20.29	3.34	56.54	15.71	0.62	0.22	0.12	3.22
pCHK966-7	0.04	1.43	25.51	2.69	59.72	8.47	0.48	0.23	0.08	2.73
pCHK966-8	0.05	1.54	22.18	3.26	57.83	12.86	0.55	0.24	0.00	2.77
CHK22 Control	0.04	1.50	26.06	3.13	59.36	7.76	0.46	0.32	0.00	1.58

Example 26: Expressing *Chlamydomonas eustigma* LPAAT2 (CeLPAAT2) in *P. moriformis* strain CHK22.

[0280] *CeLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK970 contained 5' and 3' homology arms to permit targeted integration of *CeLPAAT2* into the genome and is shown in **Table 53**.

Table 53: Integrative sequences for the transformation of *P. moriformis* with pCHK970 encoding *CeLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
38	gctcttcgcttgcgcgaccctcgttgatctgggagccctgcgcagcccccttaaatcatctcagtcagggtttctatgttcaactgagcctaaagggc tttgcacgcgcacgacacagctatctcggctacgcagctctctcagaagcggtagaacaggttgcgaagccctcgtcggtcgaanaacttgcgcc agtactatftgaattaaatfaaatgatcgaatgagacgcgaanaacttttgcagaatgccactgagtttgcgccagagaatgggagtgagcccaatcacc atccgcttgcgcagccgctgattgcgcagacgatgaatggcgagaccaggagcgcttgcgagccccgagccggttagcaggaataacggct gacaattctctgttcaactactgcgaaccattagaaagagccggagcgcttgaaattctgaatttctcgtatgcgaatgggagctcgt gaacctaaagctcggcctatgttttaagcgcatcaagatgcacgcggccccagggcacgtgtctcaagcacaaacccagccttagtttcgaga ctttgggagatagcggcagatattctagtttggcaattttgtatatttaataagctctcgaatggagcgctctgatgcggtgcagcgtcggctgcagea cttggcagtggtgcgtgggtgcgccctatcgtcggcgccctggctcagcttggctccgcgcctcctgcggcgccgcttcttcttgcgcctatga CACITCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGGCGCTGCAITGCAACACCGA TGAITGCTTCGACCCCCCGAAGCTTCCTTCGGGGTCTGCAITGGGCGCTCCGATGCCGCTCCAGGGC GAGCGCTGTTTTAAATAGCCAGGCCCCCGATTGCAAAAGACATTATAGCGAGCTACCAAAGCCA TATTCAAACACCTAGATCACTACCACITCTACACAGGCCACTTCGAGCTTTGTGATCGCACITCCG CTAAGGGGGCGCCTCTTCCCTCTTCGTTTCAGTACAAACCCGCAAAACTGTACAATGctgctcagggct tctgttctcgtcggcttgcgcgcccgaagatcagcgcctcatgacgaacgagacgtctcgaagccctcgttgcacttacccecaacaaggg ctgatgaacgaccaccaacggctcgtggtgacgaggaaggacgcgaagtgcactgtacttccagttacaacgcgaacgcacgtctggggga cgccttctgtctggggccacgccacgtccagcagcttgaacaaatcgggagggaccagcccatcgcctaccgcccgaaagcgaacgactcggcgcc ttctccggctccatgtgtgtggtgactacaacaaacacctccggcttcttcaacgacacatcgacccgcgcagcgtcgcgtggtgccaatctgacctaada caecceggzagtcgagggacagtaactctctacagccttgacggcgcggtacaccttcaacgagtaaccagaaagaaaccccgctgtggcgcccaact ccaccagatctccgcgacccgaaggtcttctgtgacgagcctcccaagaagtggtatctgaccgcggccaagtcaccaagactacaagatcagatct actctccgacgaactgaagctcgtgaagctggaggtccgcttccccaacagggtcttctcgtgttccagttacgagtgagtgccccggctgatagggt ccccacgcagcaggaccccaagcgaagtcatacttgggtgtagtctatctcacaaccccgccgcgccggcgccggcgctcttcaaccagtaacttctgc gcacgttcaacgcaccacacttcaagccttctcgaacacacagtcgcctgtztaacttgcgaaggtactactacgcctcgaacgtcttctcaacac

[0281] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmAMT03:*CeLPAAT2*:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *CeLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *CeLPAAT2*. The initiator ATG and terminator TAG codons of the *CeLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0282] Table 54 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK970. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 54: Screen of primary transformants of CHK22 with pCHK970.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK970-1	0.05	1.50	21.40	3.08	59.49	12.02	0.57	0.32	0.11	1.58
pCHK970-2	0.05	1.55	22.86	3.06	58.95	11.22	0.54	0.31	0.00	1.59
pCHK970-3	0.06	1.62	20.68	3.30	58.71	13.22	0.59	0.32	0.11	1.5
pCHK970-4	0.06	1.63	20.85	3.07	58.65	13.27	0.60	0.31	0.11	1.59
pCHK970-5	0.07	1.90	24.20	2.98	54.59	13.64	0.66	0.29	0.09	1.59
pCHK970-6	0.05	1.58	22.46	3.12	58.84	11.58	0.55	0.32	0.10	1.57
pCHK970-7	0.04	1.55	24.70	3.03	59.21	9.23	0.52	0.30	0.09	1.54

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK970-8	0.06	1.58	20.53	3.24	58.59	13.56	0.61	0.32	0.00	1.55
CHK22 Control	0.04	1.50	26.06	3.13	59.36	7.76	0.46	0.32	0.00	1.58

Example 27: Expressing *Pedinophyceae* sp. LPAAT2 (*PedLPAAT2*) in *P. moriformis* strain CHK22 using the PmAMT03 promoter.

[0283] *PedLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK972 contained 5' and 3' homology arms to permit targeted integration of *PedLPAAT2* into the genome and is shown in **Table 55**.

Table 55: Integrative sequences for the transformation of *P. moriformis* with pCHK972 encoding *PedLPAAT2*.

[illegible]

[illegible]

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text.

The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *PedLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmAMT03 promoter, indicated by the boxed italicized text, drives the expression of the *PedLPAAT2*. The initiator ATG and terminator TAG codons of the *PedLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0285] Table 56 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK972. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 56: Screen of primary transformants of CHK22 with pCHK972.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK972-1	0.05	1.56	23.91	3.02	58.99	10.16	0.51	0.33	0.09	1.49
pCHK972-2	0.04	1.54	25.41	3.08	59.09	8.65	0.48	0.30	0.09	1.86
pCHK972-3	0.04	1.58	25.14	2.92	58.78	9.28	0.49	0.27	0.09	2.00
pCHK972-4	0.05	1.64	20.42	3.47	57.89	14.28	0.56	0.17	0.00	4.47
pCHK972-5	0.06	1.73	21.15	3.09	57.89	13.56	0.61	0.31	0.10	1.55
pCHK972-6	0.05	1.69	20.40	3.21	57.44	14.85	0.57	0.21	0.00	4.33
pCHK972-7	0.06	1.68	20.59	3.45	57.50	14.43	0.57	0.22	0.12	4.16
pCHK972-8	0.05	1.63	24.13	3.09	58.15	10.67	0.52	0.27	0.09	2.09
CHK22 Control	0.04	1.50	26.06	3.13	59.36	7.76	0.46	0.32	0.00	1.58

[0286] As the transformants of CHK22 expressing CrLPAAT2 produced the higher OP:OO ratio, the percent identity (%) of all the amino acid sequences across the entire LPAAT protein was compared against that of CrLPAAT2 the results are shown in Table 57. Pairwise identities (%)

between all the sequences vs CrLPAAT2. The resulting phenotype of expressing these LPAATs in CHK22 is indicated with the m/z (577.5/603.5) OP:OO ratio; the OP:OO ratio shown in the rows of PmLPAAT1 or PmLPAAT2 represents the analytical results of the non-transgenic line CHK22.

Amino acid sequences of the LPAATs described herein are listed in **Table 58**.

Table 57: OP:OO Ratio

Gene Name	Organisms	GenBank ID	Identity (%)	m/z (577.5/603.5) OP:OO
CrLPAAT2	<i>Chlamydomonas reinhardtii</i>	XP_001699586.2	100	3.48
VcLPAAT2 V2	<i>Volvox carteri f. nagariensis_120491</i>	XP_002947580.1	34.36	1.59
AgLPPAT2	<i>Astrephomene gubernaculifera</i>	GFR47784.1	69.10	2.21
EdLPAAT2	<i>Edaphochlamys debaryana</i>	KAG2489453.1	59.94	3.78
CeLPAAT2	<i>Chlamydomonas eustigma</i>	GAX78532.1	55.91	1.59
DsLPAAT2	<i>Dunaliella salina</i>	KAF5838861.1	44.85	2.35
PedLPAAT2	<i>Pedinophyceae sp</i>	CAG9465508.1	44.61	4.47
SceLPAAT2	<i>Scenedesmus sp. NREL 46B-D3</i>	KAF6259536.1	39.76	1.64
McLPAAT2	<i>Micractinium conductrix</i>	PSC72189.1	39.76	1.55
ChsoLPAAT2	<i>Chlorella sorokiniana</i>	PRW57325.1	40.73	1.53
ChvaLPAAT2	<i>Chlorella variabilis</i>	XP_005852243.1	39.21	1.58
RsLPAAT2	<i>Raphidocelis subcapitata</i>	GBF93205.1	40.06	1.58
ChdeLPAAT2	<i>Chlorella desiccata</i>	KAH7617329.1	40.30	1.71
ApLPAAT2	<i>Auxenochlorella protothecoides</i>	XP_011401335.1	37.05	1.58
ChprLPAAT2	<i>Chloropicon primus</i>	QDZ21586.1	34.13	1.62
AGPAT1	<i>Homo sapiens</i>	NP_001358366.1	11.32	3.60
VrLPAAT2	<i>Volvox reticuliferus</i>	GIM16764.1	80.77	1.69
ChvuLPAAT2	<i>Chlorella vulgaris</i>	KAI3438340.1	39.21	1.62

Table 58: LPAAT Amino Acid Sequences

SEQ ID NO:	LPAAT	Amino Acid Sequence
40	VcLPAAT2 V2	MSVPSKALS LPSFLFS VFV FYWSLPLFAIIYRIRFANVGKRNDML DWAKALVAFFRVTVLKQGEMSLYRGGRCLYLCNHRSWADFFI DTYLTEGRAALMSRWMVFFAFPVFCTSVLILRGIVLFKRGHIAD KETASLQPIEEAFNVWLDATLRKSTVPGLLVYPEGHRSLKPRSLP LKRGMLHFASRHLAVQLIITRGKEEVIKPTDFDSFDAFFQEVQT TWDACWQLSYHQPTGVPRLSLKDAHEYDYPPSMWWLQLGVT LLSIVLLAAVLYGSWLVL RAGILSLGSSGTQQTHRYVSDVPDLD QLTALQEFMGLPGDVDLQDYADVPERMRTLVLDLVVGRTTEESA KGCCQKKRAVFEISRYRDY AALCTEGSQVMHALDGVITDLSNG

SEQ ID NO:	LPAAT	Amino Acid Sequence
		TCDESTESVERISALPAEGVGETGVEGARLLNYISFFGTPLGEGA WQACRTVMDRRARAASILDEPFTLEEVARAVGGPLNGKAHGLE VAPSECYRYARLPRVDEQGRQGTEVNCIVPVLHVLLAHIRTTGD FPQQFTKTVVSPILKKGDPLVAGNYWGIAVGGAFACYASVILN RLVRAGETLDRHPCQAGFRLGFHTAHLFVKQHLVRRRRSGG CPALRCRRLPRR
41	AgLPAAT2	MLCLYRSVIFILSSSVLAFICHFLGALGACVLWGQLSSRNLF GLRCNCCLLTDSMEASSKVLTFPAFLCSVFVFWYSLPIFAIYRIR FANLGKRNDMLEWAKSLVRFFGVTVLKEGESSLYRGGKCLYLC NHRSWADFFIDAYLTEGRAALMSRWLVYFVFPVFCTSCILIRGI VLFKRGTIADKEAFNSWLDATLGRSHVPGLLVYPEGHRSLKPAS LPLKRGMLHYAFSRKIPVQIIITRGKEEVLSEKRMVRFGRTLVT QFSKVIEPGQFASFDAFFSAVQSTWDECWRGAYGASTTSTPRHT LSHPPTEYDYPARMRLQQAATLFSMVVLAVLAGSWRAGAAV
42	EdLPAAT2	MAVSKLLGLPAFLFSVFAFYWSLPLAITTYRLRFLNLGKRNDML DWGRGLVNFFAVTIYKVGDSLKGGSCLYLCNHRSWADFFID IYITEGRGAMMSRWLVFFVFPVIFCSAVVTLKGILFKRGTIADKEK FNAWLDATLASSPVPGLLVYPEGHRSLKPHSLPLKRGMLHYAFS RKLPVQVIITRGKDDVLSEKTMVHWRRLVTSYGVKVPSECA SFDAFMHEVQTEWDKHWAEAYNAPKPPSKSLPRLVPAPHGYD YPLSMRLGMGSVTTFSIFAFAGVLYGTYCTALWVLAFTGAAQK VIVTVLALWMGLALTRAVL
43	DsLPAAT2	MGQPLLQKFISLPTFLLAIFLLYWSLPICVLVGTCLKLLDFTFPRTD MYNWCQFMTKLFRIKIKKYGPNGLRKDTGRHILYLCNHRSWTD FFLDAYLTEGNGSLMSRWLVFAVFPVFMVAVIVLRGVILFKRGT VLDKEKFNEWLDSKVSSSPIPGMLVYPEGHRSLAHKSLPLKRGML LYYAHSRKFPVQIIISRNKEEVLSEKTMKAHFATIVTGFSELIES EGKDIKEFLSEVQTAWDKMWDSVYSADAAALPELHTDVPKFD YSFSMRVMQLLVVSGTVVFLGTMAAMVKVCVCLAATGDLG CKVLVSSLVGWFLVSILNSRAPSGK
44	SceLPAAT2	MLLILKLPALLAVYVMYWSLPLANLLFIKAWKVVGNRNDM YRWCQFLVRACGIKVRKCGKQELYRGGACLYLANHRSWADFF VDAYLTEGRGQLMSRAAVIFVFPVILPLTLVRGCICFRDGVK DKEKFNRWIDACIGASPPGICLFPEGHRSLPHSLPLKRGMLNY AYSRKMPVQIVISAHKELAMDEKSFHVRFGATCVTGYGPLMRT ADYASFDEFYALQGEWQKLWVEVFSADPAALPELVISAPAGFT YPLQLKLSQLLLTLLELALMAWGLAWSVRAWGGVAARLAGTL QLGVSGLQVLQYLPLVWLVSLSASYDSTLPQPVLAAADGTPLV GSSQATRDSKKAL
45	McLPAAT2	MRLGRAIARACRWPTFLGSILLYWSVVCVALSYHFKWLNLRG RRNDVLGWSSGMKWWVRKVLKVGDRLEYRGPVYLFNHR WGDFIVDQYATEGRSLFMSRLTVLFVFPFMGALKVLKSVITFK RGAIADKERFNNWIDEQRAASPQTGLSVYPEGHRSTAPVSLPLK RGMLYYAHSRRLPVQVIIGANKEAIISEKHCTARLNQTAAVGYS

SEQ ID NO:	LPAAT	Amino Acid Sequence
		DPIMTADYDPDFEAFWEQVQEAWNKTWAEVYGADWEGLPELAP GSAGWQDPDTHYPLSVTLPMVGVVLNFAAFAGVSWLAFRAL SALFALFGPAQPLALALVAAYLALSFYLYSRPVDALQLHERLRR ESKHPSHLARSAHLAGPRGVDANGAAAAEARVAALRHARPT HRPTPYLGRRRPLAAPPARAPPRLSLRAPSAATAVARGML
46	ChsoLPAAT2	MSVPQLVAKACRWPTFLGSIILLYWSVAFITLSGHLKWWRLRGR RNDVLAWSAGMKWWVRCRVLKVGSTDLRQPCVYLFNHRSW GDFIVDQYVTEGRSLFMSRLAVLGVFPTFIYSLVILKSIILFKRGS ADKQRFNAWIDKQREGSPQTGLAVYPEGHRSTLGEPLPLKRG LHYAYSRLKLPVQIVIGANKEAIIAEKRCTARLGQTAVVGYSEPIIT ADYDPDFEAFWAEVQEAWDAQWQAVFGADWQGLPELAVQEPV THYPLSITLPMGLMTWLNGLFWTCWLTFRVVRWAFALFGPA QPLALAAAAAYLALSFWLYSQPVNALKAMQTORGHGPKLAES EANLAALDGSGARRDS
47	ChvaLPAAT2	MTLARLIAKATKWPTFLGSILLLYWSVVFVALSYHIKWLKLKGR RNDVLGWAAGMKWWIRCRVLKVGQTQELYRGQCXYLFNHRSW GDFIVDQYVTEGRSLFMSRLAVLGVFPTFVSALVVLKSIILFKRG SVADKEKFNRWIDEQRAASPPGLSVYPEGHRSTLGEPLPLKRG MLHYAFSRKLPVQIVIGANKEAIISEKHCTARLNQTA VVGYSAPIMT ANYPSFDAFWEEVQEAWNAQWAAVFGADWDGLPELAPQEPDT EYPASITLGMGLGVVANMALFCLTCWLSFRFWRWLFALFGPAQ PVALAAAAGYLALAFWLYSRPVNALQLHAEKRPPGQLAQSAT NVAALDGKKAS
48	RsLPAAT2	MLLLRLPAFWLAVWL MYWSLPLGVLTLYIPVWQLWGARNDTF YWVKFLVTALGIKFRKRSGELYSGQAPCIYLANHRSWADFFV DAYLAGGRAQMLSRMAVLYAFPMFMAPVIAMRG CIVFKRGRID DKEAFNKWIDGLVEASPQNGLMVYPEGHRSTLAHSLPLKRGML HYAYSRLKMPVQIIMSANKESVLSEKELHVGFGCTVVTSSYGLISP GDYAGFEGFMAAVQAAWDKWRDTMSADAAGLPPLHVSGTID DNHYPPRLVVSQ LFFSLLGITLLFAASSITWGWILRAKGVLPQG AKMVVSFLVAWFAASLAYCYNGQLPKPRLLPDGSEANGGGAG WKAGGRPGGGAAAKGGALKAAAAGGGGGGSPKKSS
49	ChdeLPAAT2	MSIPKALASALRWPPFLFSVFCLYWDVAILVLT YRIKWLD FKKK RNDVLALGKGLKDIHRVTILKVG YRELYRKNCMYLCNHRSWA DFLVDQYVTEGRTLFVGRWAVAAAFPLVMIPLRAIRCAILFKRG HIADIEGFNKWIDSQFANSPQTGLGVYPEGHRSTHGESLPLKRG MLKYAFSRKLPVQIVIGGNKESILSEKHCTARFYQTIA VGFSEVL KPEEYDDFENFMQKVQETWNREWSEVFSSNLKGLPVLPEVREP QLDYPMDVRMIMAICAVVNILMLAGVAWLTWWAVAAVMAL MGPLQWPVAGLVVA YIVASFYVYSKADDVRRTHQKMFQQRKQ APPVISAAGAGEGAGEGAGEENGDKKEQ
50	ApLPAAT2	MSLQRTLDSALRWPAFLLSL FILFWSVPFCSLTYYIRFLDLKTER RGDVFSWAVGMKRWFGIKILKVG DRELFRKDCMYLINHRSWG DFLVDQYLTEGRSTFLARAMVAVAFPPFMTVIMVMKATMVFK

SEQ ID NO:	LPAAT	Amino Acid Sequence
		RGNIPDKEKFNRWIDSEFAATPQTAIAVYPEGHRSTMTDSLPLKR GMLHYAYSRLKLPVQVIIAANKEAVLSEKHSTARFGQTIWAGYSE PMFPEKYETFDKFMMDKVQATWNEEWNTVFGTKPEGLREL VCE DPKLLTPLHVRRGVSTIIAVVDICFLAIMYYSLRMARRVSAALGP LEAPLLALLAVYVTASLVRYSRPYDGVAAASARKGKLAQASATQ APPAPKLAASGVADVSAATINRAASLAEPGDPQGRQ
51	ChprLPAAT2	MKAIKDKMSFLFAMLIIFYWSFPLTVAFGTDWLSLSYKARNDIFFW TAFLLGFFKVRTYRIGEQQCYRDGSCIWLCNHRDWGDFIDLYV VEGRACFLSRLMVLIAPVFGFYGLVTRTAVFFRRNTKGRDFTK FNQFISGQIQRSPCQGIVVYPEGTRNIKPESLPLRRGMLRYAYSER TPVQIVITTNKEHVISQKMQRASFGTKCISGFSEVIRPGDYSNFED FFDEINKTWKAEWKRTYGASISNAVEVTPSEMPLYIPFYNIKLG QYLSMMAYSIFTVALMVGVASFSFVLTQSMGLGRVVHFGGFST DCIVVSIFLGVFVACTKLITRKQD
52	AGPAT1	MDLWPGAWMLLLLLLFLLLFLLPTLWFCSPSAKYFFKMAFYNG WILFLAVLAIPVCAVRGRNVENMKILRLMLLHIKYL YGIRVEVR GAHHFPPSQPYVVVSNHQSSDLLGMMEVLPGRCVPIAKRELL WAGSAGLACWLAGVIFIDRKRTGDAISVMSEVAQTLLTQDVRV WVFPEGTRNHNGSMLPFKRGAFHLAVQAQVPIVPIVMSSYQDF YCKKERRFTSGQCQVRVLPVPTEGLTPDDVPALADRVRHSM L TVFREISTDGRGGDYLLKKPGGGG
53	CeLPAAT2	MSIPTLLSIPYFAACVFVFYWSNTLGCLCYKIKFMNLIGPRKDMH DWSEFMNWFFGVQYRQVGEQSLHKGERCLYLSNHRSWADFFV DIYLTEGMAAPMSRNLVYFVFPFMTSVSILKGIILFKRGVIEDK QKFNAWLDSKLDSSIRPSLLVYPEGHRSTRASSLPLKRGMLHYA HSRKLPIQIIMTKGKEGVLSEKHMRA SYGVTLATGFSEVIKSSNF PEFEAFALKVQQVWD AKWQEVYTADISRRPDC
54	PedLPAAT2	MNLAFPLAVAVMYWSLPIGALLYRIRFLNVFGPRNDMLEWAEM MRAFLGVRFKLVGQRRLLHKGERMVYLANHRSWADFFLDVWTT EARCSIMSRWMVFFAFPVFMA SVIVLRGIVLFKRGKIKDKDAFN AWLDKTLDRQVNSGSLLLYPEGTRNTKPHALPLKRGMLKYSWS RGLPFQIIVTTNKERVLSEKKMAVKFGVTCCTGFSEVIESKRYKD DFDGFMEEVQRVWDKEWAEVYKAAAEPEKLEDLTIPSPDGF DY LPSMKLLQVLVTLTIAVFSAMVVG SAYALGLVVPAAQDAWK YASYVLASMPILGLASKLLGGSKVGAIKYPGLASKKID
55	VrLPAAT2	MSVISKALSFP SFLFSVFVFYWSLPLFAIIYRIRFMNVSKRNDMLD WAKALVAFFRVTVLKQGEMTLYRGARCLYLCNHRSWADFFID TYLTEGRAALMSRWLVYFVFPVFCTSVIILRGIVLFKRGHIANKE AFNAWL DATLAKSTVPGLLVYPE
56	ChvuLPAAT2	MPVPRIVAKATRWPTFLGSILLFW SVVCVALSYHIKWLKLRGK RNDVLGWAAGMKWWIRCRVLKVG SQDLYREKCVYLFNHRSW GDFIVDQYVTEGRSLFMSRMAVLFVFPFTFISALTVLKSIILFKRGA IADKEKFNAWIDEQRANSPQSGLSVYPEGHRSTKRDSLPLKGRM

SEQ ID NO:	LPAAT	Amino Acid Sequence
		LYYAFSRKLPVQIVIGANKEAIISEKHLTARLGQTAVVGYSEPIM TAEYADFDFAFWAKVQETWDRQWQTVFGAAWEGLPELEPVEPD TEYPLSITGRMVLVVIANLLLFCCACWLTFHFWTWLFALFGPLQ PAALALSATYLALALWIYSQPVNALQVHAKKLPRGPQAQPAVD MAALLETKKAS

Example 28: Expressing *Volvox reticuliferus* LPAAT2 (*VrLPAAT2*) in *P. moriformis* strain CHK22.

[0287] *VrLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1133 contained 5' and 3' homology arms to permit targeted integration of *VrLPAAT2* into the genome and is shown in Table 59.

Table 59: Integrative sequences for the transformation of *P. moriformis* with pCHK1133 encoding *VrLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
57	gctcttcgcttgcgccacccctcgtgatctggagccctcgcgcagcccttaaatcatctcagtcaggtttctatgttcaactgagcctaaagggtcttcgac aogcgcacgagcagacgfatatcggctacgcagctctcagaagcggtagaacagttcgcaagccctcgtcggcgaaaacttgcgocagttactatgaaat taaatataatgattgaaatgagacgcgaaacttttgcagaatgccacigaggttgcgccagagaatggagtgccgocaitcaecatccgctgtgcacggcct gattcgcgcagacgaggaatggcgagaccaggagcggcgtgcgagcccgagccggtagcaggaataacggcgcacaatcttctgtccaatactgg caaccattagaagagccggagcgcgttgaatcttgcacatcagtaattttcgtatgcggcgccctgctgaacctaaaggctccggcctatgttitaaggc gatccaagatgcacgcggcccgagcagcgtgtctcagaacacacccagccitagtcttgagacatttgggagatagcggcagataatcagtttggcatttt gtatattatgaatgctcgaatggagcgcctcgtatgcggcgcagcggcgtgcagcagccctggcagtgccgctggggtccctctatcgtcggcgcctgg teagctggctcccgccctcgtcggcgccctCTTTCCTTGGCGCTATGACACTTCCAGCAAAAGGTAAGGGCGGGCT GCGAGACGGCTTCCCGGCGCTGCATGCAACACCGATGATGCTTCGACCCCCGAAAGCTCCTTC GGGGCTGCATGGGCGCTCCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCC GATTGCAAAAGACATTATAGCGAGCTACCAAGCCATATTCAAACACCTAGATCACTACCACCT TCTACACAGGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTT TCAGTCACAACCCGCAAACTGTACAATGctctgcagggccttctgttctgtctggccggttcgccccaagatcagcgcctc catgacgaacgagacgtccgaccgccccctggtgcacttcaccccccaacaggcgtgatgaacgaccccaaggcctgtgtacgacgagaaggac gccaaatggcaactgtacttccagtaacacccgaacgacacccgtctgggggagcccttgtctggggccacgccacgtccgacgacctgaaccaatgg gaggaccagccatcgccatcgcgccgaagcgcaacgactccggcgctctccggctccatgggtgtggaatcaacaacacccctccggctcttcaac gacacacatgaccgcgcagcgcgtgctggccatctggacatcaaacacccggagtcggagagcagtaacatcttacaacctggagcggcgccia caacttcacccagtaecagaaagaaaccccgctcggccgccaactccacccagttccgcgaccggaaggctctctgtgtacgagccctccagaaaggat catgaccgcggccaagtcacaggaactcaagatcgagatctactctccgacgacctgaagtcctgggaagctggagtcgcgttcccaacgagggtt ctcggctacagtaagtggtgcctccggtatcgaggtccaccagcagcgaacccagcaagtcctactgggtgatgttatctccatcaacccggc gccccggcggcggtccttcaacagtaacttctcggcagcttcaacggcaacccacttcgaggccttcgacaaccagtcctccggtgtgtgacitcggca aggactatcagccctgcagacettcttcaacacccgacccgaactaogggagcgcctcggcatcgggtggccctccaactgggagttactcgccttcgt gcccacacacccctggcgctctccatgtctctcgtgcgaagtctctcacaacccgagtaaccagccaccccgagacggagctgataacccigaa ggccgagccgcatcctgaacatcagcancgccggccctggagccgggttcgccacacacacccgttgacgaaggccaacagctacacagtcgacctgt ccaacagcaccggcaccctggagticgagctgggtgtacgcgctcaacacacccagacgatcccaagtcggttcggcgacctctccctcgtgtcaa gggcttggaggaccccgaggagttactcgcgtgggttcggaggtgtccgcgtctctcttctcctggaccggggaacngcaaggtgaagttcgtgaa ggagaacccctacttcaacacccgatgagcgtgaacacacccgcttcaagagcgagaacgacctgtctactacnaagggtgtacggcttgcgtgacca gaacatcctgagctgtaacttcaacgagcgagcgtgtgtccacacacacttactatgacacccgggaacgcccctgggctccgtaacatgacgacg gggttgggacaacctgtctacatcagcaagttccaggtgcgcgaggtcaagtgactTAAGACGCCCCGCGCGCGCACCTGACC TGTTCTCTCGAGGGCGCCTGTTCTGCCTTGGCAAAACAAGCCCTGGAGCATCGCTGATGATC GTCTCTGGCGCCCCGCGCGCGGTTTGTTCGCCCTCGCGGGCGCCCCGGCCGCGGGGGCGCATT GAAATTGTTGCAAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGTTGAGATGGAG GTACAGGAGTCAAGTAAGTAAAGTTTATATGATAACTAACAACAAAGGGTCGTTCTTGGCC

SEQ ID NO:	Nucleotide Sequence
	AGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGCTTGCCATCGAATGTGAGCGG GCGGGCCGCGGACCCGACAAAACCCCTTACGACGTGGTAAGAAAAACGTGGCGGGCACTGTCC CTGTAGCCTGAAGACCAGCAGGAGACGATCGGAAGCATCACAGCACAAACCGGtcagcagcagctog gatagtatc gacacactctggacgctggcgtgtgatggactgtgcgccacacttgccttgacctgtgaataicccctgccgtttatcaaacagcctc agtggttgatctgtgtgtacgcgttttgcgagttgtagctgctgtgtgtatttgcgaataccacccccagcatccccttcctcgtttcatatcgtttgcac ccaaaccgcaacttaictacgtgtcctgctaiccctcagcgctgctcctgctcctgctcactgcccctcgcacagccttggttgggctccgctgtatttcc ggtactgcaacctgtaaaccagcactgcaantgctgatgcacgggaagttagtgggatgggaacacaaatggacctagggaataacaata AAGCTT GCCTGCTCAAGCGGGCGCTCAACATGCAGAGCGTCAGCGAGACGGGCTGTGGAGATCGGGA GACGGACGAGGCCGCCCTTGCCCTGTTTGAACCTGAGCGTCAGCGCTGGCTGGCTAAGGGGAGGGAG ACTCATCCCCACGCCCGGCCAGGGCTCTGATCCCGTCTCGGGCGGTGATCGGGCGCGCATGA CTACGACCCAACGACGTACGAGACTGATGTCCGGTCCCGACGAGGAGCGCCGCGAGGCACTCC AGGGCTGCCGACCATGTTTACACCGACCAAAAAGCACTCGCTCGCATCCATTTCCGTGCGCCCGC ACATGCATCATCTTTTGGTACCGATTTTCGGTCTTGTTTTACCCCTACGACCTGCCTTCCAAGGT GTGAGCAACTCGCCCGGACATGACCGAGGGTGATCATCCGGATCCCCAGGCCCCAGCAGCCC CTGCCAGAATGGCTCGCGCTTTCCAGCCTGCAGGCCCGTCTCCAGGTCGACGCAACCTACAT GACCACCCCAATCTGTCCCAGACCCCAAAACACCCCTCCTGCCCTGCTTCTCTGTGATCGCTGAT CAGCAACACCCGGGGAATTC ATGagcgtgatctcgaaggccctctcgtttccatectttctgttcagcgtgttcgtttactggtcgt gcgcgtgtttgcgacatttacgcacccgtttcatgaacgtgtcgaagcgcaacgacatgctgactggcggaagcgctcgttcgtttccgcgtcacc gtgtggaagcaggcgagatgacctctaccgcgcgcgcgatgcctgtacgtgtgcaaccacggagctggcggaattttcatcgacacctacotga cggaggcgccggcgccctgatgtcgcgtggcctgtctactttgtcttcccggtgtgtctgcacctcgtcattatcctgcgtggatcgtgtgttcgaagc tgggcatatcgccaacaggagggcctcaacgcgtgtgctcgaagccacgtggccaaagtcacgggtgcggggccctcgtgttaccctcagTAgac gcccgcgcggcgacctgacctgtttctcgaaggcgctgtgtctgtccttgcgaanacagccctggagcatcgtgtcatgctgtctgtggcgcccg cgcgcggtttgtcgcctcgcggcgccgcgcggcgccggggcgccattgaaattgttgcgaacccacactgacagattgagggcccgaggcaggagc gttgatgagaggagacaggagtcnaagtaacigaaagtattatgataactaacaaagggtcgtttctgccaagcaatgacaagaacagattccacatt tcctgttagaggttgcacatgaatgtgagcgggcgggcgccgcggacccgacaaaaccttacgacgttgtaagaaaaacgttgcggcgcactgtccctgt agcctgaagaccagcaggagacgacgcggaagcatcacagcacaggccggcccgctcgcgaacagagcgcgccggcgccgcatcgatacctgcaggga agcgtctgcgtgttggagctggagtcgtgggttgacgacggcgctgcagctcttcaggatgtccctggcggtgcggttcacgctgtgtgagaaat atggcgacgaanagtgacggctggggcgccggcggtgtgtagccatacgcattgggtacgcggccattggatgggattgttaggtctatggaggat aatagagttttgtcggatccacgcattgtgatgcagtagcccggtgtgtgnaagtatggaggatgtgcatgtgctaticacatgcgtgccaccctt ttcgaggaatgttgcggcgcacgtgtgtgcaccgatgggnaantcgaagttcgaccactacatgaaatgtatacctctgagatgcagcaactgcggg tgcgaanaggataacggttggtaggtgtatgtcgcagcatgtgtgtgatttgcggcaactccccctgccacggcccttgcaggtgtcatgtgactgg aggatacgaacttgcggcgtaaatccagaggaggaacccctctggggcgacattgtgccctgtctcctgtgtgtgtgaaattgt

[0288] The construct can be written as

5'DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmACP:VrLPAAT2:PmPGH::3'DAO1b.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *VrLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. An acyl carrier protein promoter from *P. moriformis* (PmACP), indicated by the boxed italicized text, drives the expression of the *VrLPAAT2*. The initiator ATG and terminator TAG codons of the *VrLPAAT2* are indicated by uppercase, bold italics, while the

remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0289] Table 60 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK1133. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 60: Screen of primary transformants of CHK22 with pCHK1133.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1133-1	0.04	1.13	21.73	3.26	62.52	9.06	0.54	0.28	0.08	1.59
pCHK1133-2	0.03	1.14	23.45	3.14	61.66	8.30	0.55	0.28	0.08	1.63
pCHK1133-3	0.03	1.07	21.91	3.17	62.82	8.72	0.55	0.28	0.08	1.59
pCHK1133-4	0.03	1.12	22.63	3.20	62.35	8.40	0.52	0.28	0.08	1.53
pCHK1133-5	0.03	1.11	22.07	3.04	62.71	8.72	0.54	0.27	0.08	1.59
pCHK1133-6	0.04	1.14	22.35	3.06	62.05	9.04	0.56	0.28	0.09	1.46
pCHK1133-7	0.03	1.21	26.33	3.04	60.23	6.93	0.53	0.25	0.07	1.54
pCHK1133-8	0.03	1.13	23.45	3.06	61.89	8.14	0.53	0.28	0.08	1.57
pCHK1133-9	0.04	1.36	27.75	2.64	56.65	8.95	0.61	0.24	0.07	1.69
pCHK1133-10	0.03	1.14	24.06	3.13	61.71	7.70	0.52	0.28	0.07	1.56
CHK22 Control	0.03	1.23	26.94	3.06	60.23	6.39	0.50	0.23	0.06	1.52

Example 29: Expressing *Chlorella vulgaris* LPAAT2 (*ChvuLPAAT2*) in *P. moriformis* strain CHK22.

[0290] *ChvuLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1134 contained 5' and 3' homology arms to permit targeted integration of *ChvuLPAAT2* into the genome and is shown in Table 61.

Table 61: Integrative sequences for the transformation of *P. moriformis* with pCHK1134 encoding *ChvuLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
58	gctcttcgcttgcgcacccctcgttgatctgggagccctgcgcagcccttaaatcatctcagtcagggtttctatgttcaactgagcctaaagggtttcgtc acgcgcacgagcacacgtatateggctacgcagtcctcagaagcggtagaacagttgcgaagccctcgtcggcgaacttgcgcagctactatggaat taantfaaatgacgaatgagacgcgaactttgcagaatgccactgagtttgcgcagagaatgggagtggcgcattcaccatccgcctgtgcacggcct gatcgcgcagacgatgaatggcgagaccaggggagcggcttgcgagcccccagcggtagcagggaataacggcgcacantcttctgtccaaactactgg caaccattagaaagagccggagcgcgttgaaattotgcaatcgagtaattttcagtcggcggcctgctgaaccctaaggctccggcctatgtttaaggc gatccaagatgcacgcggcccccaggcagcgtgtctcaagcacaaccccagccttagtttcgagacttgggagatagcggcagatctagtttggcatttt gatatattaattaagctcgaatggagcgtctgagcgggtacagcgtcgcgtgcagcacctggcagtgccgttgggtcgcctatcgtcggcgcctgg

[illegible]

SEQ ID NO:	Nucleotide Sequence
	atgacaagaacaagattccatttccgtgtagaggcctgccatcgaatgtgagcggcgccggccggacccgacaaaacccctacgacgtggttaagaa aaacgtggcgggcactgtccctgtagccctgaagaccagcaggagacgacatcggaagcaccacagcagggccggccggcgtctcgaacagagcggcg cgccggcaccatcgataccctgcaggaaagcgtctgtgtgtggagctggagctgtggccttgacgacggcgctgcagctcttcgaggatgtgctggcgtgc gcgttcacgtcgtggtgagaantaatggcgacgaaacgttgacggctggggccggcgggcgtgtggtgacccatagccatgggtaacggcccatgggatg ggatgggtaggcctatggagggaataatagattttgtcggatccaacgcattgtgagcagtagcccggtgtgctgaaagtatggaggatgggtgcatggc tattcacatgcgctgccaccoccttttcgaggaaatgtgccggcaccgtgtgtgacaccgattgggaaaatcgacgttcgaccactacatgaaatgtatc ctctgaagatgcagcaactgcgggtgcgaaacggataacggtttggtaggtgtatgtcgcagcatgtgctggtatttgcgggcaactccccctgccacggc ccattgcaggtgtcatgtgactggaggatagaaacttgcggcgtaaatccagaggaggacccctctggggccgacattgtgcccactgctctccgc ttgctgttccctgtgtgaaatgt

[0291] The construct can be written as 5’DAO1b::CrTUB2:ScSUC2:PmPGH:CvNR:PmACP:
ChvuLPAAT2:PmPGH::3’DAO1b. Proceeding in the 5’ to 3’ direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the DAO1b locus via homologous recombination, the *C. reinhardtii* β-tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *ChvuLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmACP promoter, indicated by the boxed italicized text, drives the expression of the *ChvuLPAAT2*. The initiator ATG and terminator TAG codons of the *ChvuLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 DAO1b genomic region indicated by bold, lowercase text.

[0292] Table 62 shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK1134. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 62: Screen of primary transformants of CHK22 with pCHK1134.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1134-1	0.05	1.33	25.28	3.25	58.77	8.99	0.53	0.26	0.06	1.63
pCHK1134-2	0.03	1.15	24.68	2.96	61.25	7.70	0.52	0.25	0.07	1.61

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1134-3	0.04	1.12	21.46	2.93	62.37	9.76	0.57	0.25	0.08	1.49
pCHK1134-4	0.03	1.16	24.92	3.05	61.37	7.26	0.51	0.26	0.07	1.55
pCHK1134-5	0.03	1.25	26.47	3.00	59.93	7.10	0.50	0.24	0.07	1.6
pCHK1134-6	0.04	1.16	22.29	2.94	61.31	9.94	0.55	0.23	0.08	1.51
pCHK1134-7	0.04	1.19	22-15	3.00	61.86	9.39	0.54	0.27	0.08	1.58
pCHK1134-8	0.04	1.16	22.78	3.11	62.01	8.59	0.54	0.27	0.08	1.62
pCHK1134-9	0.04	1.21	21.83	2.81	61.24	10.47	0.58	0.24	0.08	1.52
pCHK1134-10	0.04	1.20	22.03	2.95	61.11	10.32	0.55	0.24	0.08	1.56
CHK22 Control	0.03	1.23	26.94	3.06	60.23	6.39	0.50	0.23	0.06	1.52

Example 30: Generation of a classically improved microalgal strain that produces a triglyceride oil enriched in palmitate.

[0293] The microalgae *P. moriformis* strain CHK22 (UTEX 1533) was subjected to classical strain improvement to improve productivity, yield on carbon, and increase palmitic acid content. The mutagenesis, trait selection, and high-throughput, automated screening steps of the improvement process are outlined in the flow diagram in **FIG. 9**. Briefly, algal cells in the log phase of growth were subjected to mutagenesis by treatment with chemicals or UV light (1). Cells were then sub-cultured into a lipid production medium and subjected to selection/enrichment strategies (2). Strains were then plated to a solid medium to obtain clonal isolates (3), followed by the interrogation of these isolates in a lipid production medium in a 96-well format (4). Using glucose consumption as a surrogate for oil production, high glucose consuming strains were validated in a lipid production medium in a tube or shake flask format (5). Successfully validated isolates were sub-cultured for multiple generations to stabilize mutations (6), followed by purification of clonal isolates (7), and subsequent re-interrogation in lipid production medium (8). Phenotypically stable clones were then validated in fermentation (9). Clones showing variability in (8) were revalidated and sub-cultured via (6) to generate stable lines.

[0294] CHK22 was chemically mutagenized for 30 min at 32 °C with 44 µM 4-nitroquinoline 1-oxide (4-NQO) or subjected to sham mutagenesis by the addition of only the mutagen solvent, DMSO. The mutagen was inactivated with the addition of sodium thiosulfate, which was then removed with repeated washing with water. The cells were then allowed to recover for 3 days in limited sugar growth media. The mutagenized and the mock-mutagenized populations were then independently cultured in lipid production media at 38 °C for five days. An optimal lipid production temperature for CHK22 is typically between 28-32 °C. A higher-than-optimal

temperature was applied as a stressor to provide a growth advantage to mutants having a predisposition toward producing higher palmitate levels. At the end of the 5-day, 38 °C lipid culture, the cells were collected and then incubated at 65 °C for 4 min. Based on range-finding experiments conducted in the same format, exposure to temperatures within this range for this duration of time typically kills >99% of the cell population. After heat exposure, the cells were recovered in limited sugar growth media for three days, diluted, and then plated. Mutant clones were then selected and assessed for glucose consumption rate and fatty acid profile in a 72h, 96-well block-based lipid production assay (as illustrated in **FIG. 9**). **Table 63** summarizes glucose consumption rates and fatty acid profiles of TAG oil produced by the mutant strains. One mutant produced about 8% more palmitate than the CHK22 parent strain. This strain exhibited a significant reduction in glucose consumption rate, a hallmark of lower lipid titer.

Table 63: Fatty acid profiles of select mutant strains exhibiting higher glucose consumption (Glc Rate) in a 96-well block lipid assay and increased palmitate (C16:0) levels.

Sample	Glc Rate (g/L *D)	C12: 0	C14: 0	C15: 0	C16: 0	C16: 1	C17: 0	C17: 1	C18: 0	C18: 1	C18: 2	C18: 3 alpha	C20: 0	C20: 1	C22: 0	12- OH, 9 _{omega} - C18: 1	C24: 0
CHK22 mutant-1	5.26	0.22	5.38	0.06	35.86	1.38	0.07	0.03	2.16	43.45	9.40	0.76	0.26	0.11	0.07	0.11	0.09
CHK22 mutant-2	7.11	0.04	1.68	0.04	31.02	1.00	0.08	0.04	2.70	53.39	8.61	0.54	0.26	0.11	0.05	0.13	0.07
CHK22 mutant-3	6.90	0.04	1.53	0.04	30.60	0.92	0.09	0.05	3.00	54.78	7.57	0.56	0.26	0.10	0.05	0.13	0.06
CHK22 Control	7.00	0.04	1.51	0.05	27.92	0.84	0.10	0.04	3.16	56.73	8.19	0.59	0.33	0.10	0.07	0.07	0.08
CHK22 Control	7.17	0.04	1.58	0.04	28.09	0.76	0.11	0.05	3.38	56.11	8.23	0.61	0.35	0.13	0.06	0.15	0.08
CHK22 Control	7.18	0.04	1.59	0.04	28.61	0.85	0.10	0.05	3.29	55.89	8.02	0.63	0.31	0.10	0.08	0.13	0.07

[0295] To further adapt the CHK22 mutant-3 strain to the altered fatty acid composition and improve lipid productivity, the strain was serially passaged in the absence of any selection for more than thirty population doublings and then plated to solid media to yield single colonies. These sub-clones were screened in a lipid production assay to assess the phenotypic stability of the lineage for both glucose consumption rate and fatty acid profile. After one cycle of serial passaging, the mutant was still phenotypically unstable. Several sub-clones that retained the higher palmitate level phenotype, along with increased rates of glucose consumption, were taken through additional rounds of serial passaging and stability assessment. After a total of five rounds of serial passaging and stability assessment, a stable strain, designated CHK100, was identified.

CHK100 produced about 5% more palmitate than the CHK22 parental strain. The oil content (% on a weight-by-weight basis; %w/w), dry cell weight (DCW), and lipid titer (g/L) of CHK100 were also greater than those of CHK22 (**Table 64** and **Table 65**). **FIG. 10** illustrates the strain improvement strategy of CHK22 to produce CHK100.

Table 64: Tube-based assays on CHK100 showing improved lipid titer.

Sample	Oil Content %w/w	Avg. %w/w	DCW (g/L)	Avg. DCW (g/L)	Lipid Titer (g/L)	Avg. Lipid Titer (g/L)	NLB (g/L)	PCP
CHK100-1	78.6	77.3	11.4	11.5	9.0	8.9	2.4	3.7
CHK100-2	78.0		11.3		8.8		2.5	3.5
CHK100-3	75.5		11.6		8.8		2.8	3.1
CHK100-4	77.1		11.6		8.9		2.7	3.4
CHK22 Control	77.7	76.2	8.8	9.2	6.8	7.0	2.0	3.5
CHK22 Control	76.1		9.1		6.9		2.2	3.2
CHK22 Control	76.0		9.4		7.1		2.3	3.2
CHK22 Control	74.7		9.6		7.2		2.4	3.0

NLB, non-lipid biomass; PCP, per cell production.

Table 65: Tube-based assays on CHK100 showing increased C16:0 content.

Sample	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	C21:0	C22:0	C24:0
CHK100-1	0.03	1.22	0.02	0.03	32.25	1.36	0.06	0.04	2.03	55.65	6.36	0.50	0.19	0.05	0.05	0.04	0.00
CHK100-2	0.03	1.22	0.03	0.03	32.25	1.36	0.07	0.04	2.02	55.61	6.37	0.53	0.19	0.05	0.05	0.04	0.00
CHK100-3	0.03	1.18	0.02	0.03	32.07	1.40	0.07	0.03	1.99	55.89	6.27	0.57	0.18	0.05	0.05	0.03	0.00
CHK100-4	0.03	1.18	0.02	0.04	32.15	1.38	0.07	0.03	1.99	55.91	6.25	0.55	0.19	0.05	0.04	0.03	0.00
CHK22 Control	0.04	1.39	0.02	0.03	27.59	0.85	0.09	0.03	3.27	57.92	7.45	0.64	0.33	0.07	0.06	0.06	0.07
CHK22 Control	0.04	1.39	0.02	0.03	27.62	0.85	0.10	0.04	3.27	57.88	7.44	0.63	0.32	0.07	0.06	0.06	0.06
CHK22 Control	0.04	1.36	0.02	0.04	27.39	0.85	0.10	0.03	3.27	58.28	7.30	0.68	0.31	0.07	0.06	0.06	0.06
CHK22 Control	0.04	1.36	0.02	0.03	27.37	0.85	0.9	0.04	3.29	58.34	7.30	0.67	0.32	0.07	0.05	0.06	0.06

[0296] All strains were subjected to standard lipid production conditions in which strains were grown in duplicate in 10 mL of lipid production medium. Cultures were grown with shaking (200 rpm) for 121 hours at 28 °C at which point about 1 mL of biomass was removed, applied to a polycarbonate filter, washed with an equal volume of Milli Q water, and placed in a tared glass vial at -80 °C for 30 minutes. Vials containing filters and frozen biomass were lyophilized to dryness overnight, weights were recorded, and filters with dried biomass were subjected to direct transesterification followed by GC/FID to quantitate FAMES.

[illegible]

[0298] The construct can be written as

5'Thi4::CrTUB2:ScSUC2:PmPGH:CvNR:PmACP:*PedLPAAT2*:PmPGH::3'Thi4. Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK100 that permit targeted integration at the Thi4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK100 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *PedLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmACP promoter, indicated by the boxed italicized text, drives the expression of the *PedLPAAT2*. The initiator ATG and terminator TAG codons of the *PedLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P.*

moriformis PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK100 Thi4 genomic region indicated in bold, lowercase text.

[0299] **Table 67** shows the fatty acid/TAG profiles of primary transformants of CHK100 transformed with pCHK1126. The *P. moriformis* base strain CHK100 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 67: Screen of primary transformants of CHK100 with pCHK1126.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1126-1	0.03	1.24	29.75	2.10	57.26	7.38	0.39	0.17	0.07	3.79
pCHK1126-2	0.03	1.23	30.71	2.19	55.74	7.74	0.49	0.19	0.07	4.42
pCHK1126-3	0.03	1.28	32.21	2.28	54.06	7.59	0.69	0.21	0.07	4.5
pCHK1126-4	0.03	1.24	30.53	2.22	56.36	7.40	0.42	0.17	0.07	4.31
pCHK1126-5	0.03	1.33	32.07	2.29	55.48	6.47	0.42	0.19	0.05	1.47
pCHK1126-6	0.04	1.69	31.44	2.23	55.42	6.84	0.47	0.18	0.06	4.46
pCHK1126-7	0.03	1.30	32.18	2.28	55.38	6.49	0.45	0.18	0.05	1.52
pCHK1126-8	0.03	1.24	30.45	2.32	56.35	7.40	0.45	0.18	0.07	4.72
pCHK1126-9	0.03	1.25	30.99	2.30	55.94	7.29	0.43	0.18	0.07	4.39
pCHK1126-10	0.03	1.18	29.08	1.41	57.06	8.73	0.28	0.12	0.08	4.35
CHK100 Control	0.03	1.33	32.22	2.29	55.28	6.50	0.44	0.18	0.05	1.60

Example 32: Expressing *Pedinophyceae* sp. *LPAAT2* (*PedLPAAT2*) in *P. moriformis* strain CHK100 using the PmG3PDH promoter.

[0300] *PedLPAAT2* was introduced into a *P. moriformis* strain CHK100. The expression construct pCHK1127 contained 5' and 3' homology arms to permit targeted integration of *PedLPAAT2* into the genome and is shown in **Table 68**.

Table 68: Integrative sequences for the transformation of *P. moriformis* with pCHK1127 encoding *PedLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
60	ccctcaactgagcgtgggaacctctccgggcaggcgaatgtcggtgggttgccctcttgacacggtctacacggtcgagtacgcatgagge ggtgatggctgtgtggttgccacttcgtccagagacggcaagtcgtccatcctctggtgtgtggcgagcgtgcagcagtcctctgcagcag atgagcgtgactttggccatttcacgcactcgagtgtacacaaatccattttcttaaagcaaatgactgtgattgaccagatactgtaacgtgatt tcgtccagatgcacagatagcgaccatgtgtgctgcgttcgaaatctggattccgaattcgacctggcgtccatccatgcaacagatggcga caattgttacaattcctgtcaccatcggaatggagcagggtccacttagattccgataccacgcacatctcgctaatagtcattcggtcgtgtctt cgatcaatctcaagtgtgtgtgcatggatctgtgtgacgatcggtatgggtttgcccgtgtggcgtgagggctgcccgaaggcaagctaaccca gctcctctcccgcacaatactctgcaggcaaaagccgggtcacttgccttcagattgccaaataaacctcaattatggcctctgtcatgccatcatgg gtctgatgaatggtaacgctgtgtctgacgggtcccgagcctctggggtccctgcccgcaccagcccaagcgcgcggcagtcgtgcca aggtgtgtctcggaCTTCTTTCGCTATGACACTTCCAGCAAAAAGGTAGGGCGGGCTGCGAGACGGCT

[illegible]

SEQ ID NO:	Nucleotide Sequence
	gtagtggcatgggttagaataatacacttgatttcttgcacggcaatctcggttgtecgaggttcaacccatttcggaggtctcaggtcagcc gcgcaatgaccagccgctacttcaaggacttgcacgacaacgccgagggtgagctatgtttaggacttgattggaaattgtcgtcagcatalctg cgctcgcgacagcaaccaagcaaaatgcaagtgcttccgatttgcgtcgcaggtcgatgtttgtaicgtcggcgccgagtcgcccgtctgt ccigcgttaccagctgaccaagcaaccctgacgtcgggtacgcgagctgagattcgattagacataaattgaagattaaacccgtagaaaaatt tgatggctgcgaaactgtgtcgtatgcaagaaattgatcgtctccacitccgaggtcgccalcagcagcagggcggtgtcccgccggcgcc gcttggctgggggacagctgttctcgccatgtgtgtacgtagaaggatgaatttcagctgggttctgtgcacagctgtttgtcatgattgttc agactattgtgaatgttttagattcttaggatgcattgtgtcgtcgcgact

[0301] The construct can be written as 5' *Thi4::CrTUB2:ScSUC2:PmPGH::CvNR:*
PmG3PDH::PedLPAAT2:PmPGH::3'Thi4. Proceeding in the 5' to 3' direction bold, lowercase
sequences represent genomic DNA from CHK100 that permit targeted integration at the *Thi4*
locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of
the yeast sucrose invertase gene (conferring the ability of CHK100 to metabolize sucrose) is
indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are
indicated in uppercase, bold italics while the coding region is indicated in lowercase italics.
The *P. moriformis* *PmPGH* 3'-UTR is indicated by uppercase underlined text that enables
amplification of the *PedLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics,
in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* *PmG3PDH*
promoter, indicated by the boxed italicized text, drives the expression of the *PedLPAAT2*. The
initiator ATG and terminator TAG codons of the *PedLPAAT2* are indicated by uppercase, bold
italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* *PmPGH* 3'-
UTR is indicated by lowercase underlined text followed by the CHK100 *Thi4* genomic region
indicated by bold, lowercase text.

[0302] **Table 69** shows the fatty acid/TAG profiles of primary transformants of CHK100
transformed with pCHK1127. The *P. moriformis* base strain CHK100 is shown as a non-
transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C
in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to
direct transesterification to generate FAMES for subsequent quantitation and characterization by
GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 69: Screen of primary transformants of CHK100 with pCHK1127.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1127-1	0.03	1.23	30.12	2.18	56.67	7.51	0.45	0.18	0.07	4.64
pCHK1127-2	0.03	1.25	30.36	2.41	56.39	7.34	0.44	0.19	0.07	4.15
pCHK1127-3	0.03	1.24	30.80	2.33	56.06	7.31	0.45	0.17	0.07	4.49
pCHK1127-4	0.03	1.19	29.75	2.32	57.15	7.36	0.43	0.18	0.07	4.86

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1127-5	0.03	1.27	30.60	2.33	56.14	7.36	0.45	0.18	0.07	3.9
pCHK1127-6	0.03	1.40	31.48	2.46	55.45	6.87	0.45	0.20	0.05	1.65
pCHK1127-9	0.03	1.25	30.64	2.28	56.14	7.41	0.44	0.18	0.08	5.16
pCHK1127-10	0.03	1.30	31.48	2.25	55.73	6.90	0.42	0.18	0.06	3.27
pCHK1127-11	0.03	1.21	30.09	2.28	56.78	7.39	0.44	0.18	0.07	4.89
pCHK1127-12	0.03	1.24	30.90	2.26	56.16	7.09	0.43	0.18	0.07	3.7
CHK100 Control	0.03	1.33	32.22	2.29	55.28	6.50	0.44	0.18	0.05	1.60

Example 33: Expressing *Pedinophyceae* sp. LPAAT2 (*PedLPAAT2*) in *P. moriformis* strain CHK100 using the PmL40-2 promoter.

[0303] *PedLPAAT2* was introduced into a *P. moriformis* strain CHK100. The expression construct pCHK1128 contained 5' and 3' homology arms to permit targeted integration of *PedLPAAT2* into the genome and is shown in **Table 70**.

Table 70: Integrative sequences for the transformation of *P. moriformis* with pCHK1128 encoding *PedLPAAT2*.

[illegible]

[illegible]

[0304] The construct can be written as 5'Thi4::CrTUB2:*ScSUC2*:PmPGH:CvNR:PmL40-2:*PedLPAAT2*:PmPGH::3'Thi4. Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK100 that permit targeted integration at the Thi4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK100 to metabolize sucrose) is indicated by

boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR is indicated by uppercase underlined text that enables amplification of the *PedLPAAT2* gene, followed by a linker, indicated by lowercase, bold italics, in which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmL40-2 promoter, indicated by the boxed italicized text, drives the expression of the *PedLPAAT2*. The initiator ATG and terminator TAG codons of the *PedLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The *P. moriformis* PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK100 Thi4 genomic region indicated in bold, lowercase text.

[0305] Table 71 shows the fatty acid/TAG profiles of primary transformants of CHK100 transformed with pCHK1128. The *P. moriformis* base strain CHK100 is shown as a non-transgenic control. Strains were grown for 96 hours in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 71: Screen of primary transformants of CHK100 with pCHK1128.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1128-1	0.04	1.41	34.8	1.97	49.82	8.93	0.76	0.23	0.1	3.36
pCHK1128-2	0.03	1.19	30.92	2.12	56.26	7.13	0.44	0.17	0.07	4.03
pCHK1128-3	0.03	1.17	30.62	2.16	56.48	7.21	0.43	0.18	0.07	3.7
pCHK1128-4	0.03	1.16	30.83	2.12	56.42	7.13	0.44	0.17	0.07	4.1
pCHK1128-5	0.03	1.15	30.44	2.18	56.87	7.02	0.45	0.17	0.07	4.1
pCHK1128-6	0.03	1.17	30.56	2.2	56.56	7.17	0.44	0.18	0.07	3.88
pCHK1128-7	0.03	1.18	31.27	2.05	55.99	7.1	0.43	0.16	0.06	3.92
pCHK1128-8	0.03	1.19	30.09	2.34	56.76	7.36	0.44	0.18	0.07	4.69
CHK100 Control	0.03	1.33	32.22	2.29	55.28	6.50	0.44	0.18	0.05	1.60

Example 34: Expressing *Pedinophyceae* sp. *LPAAT2* (*PedLPAAT2*) in *P. moriformis* strain CHK100 using the PmMPGp promoter.

[0306] *PedLPAAT2* was introduced into a *P. moriformis* strain CHK100. The expression construct pCHK1129 contained 5' and 3' homology arms to permit targeted integration of *PedLPAAT2* into the genome and is shown in **Table 72**.

Table 72: Integrative sequences for the transformation of *P. moriformis* with pCHK1129 encoding *PedLPAAT2*.

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direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 73: Screen of primary transformants of CHK100 with pCHK1129.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1129-1	0.03	1.43	29.85	2.41	58.08	6	0.41	0.18	0.06	4.42
pCHK1129-2	0.03	1.15	31.01	2.04	56.4	7	0.43	0.16	0.06	4.3
pCHK1129-3	0.03	1.17	31.42	2.08	56.17	6.74	0.42	0.16	0.06	3
pCHK1129-4	0.03	1.41	32.42	2.2	53.58	7.95	0.44	0.19	0.07	4.67
pCHK1129-5	0.04	1.58	31.55	2.03	55.55	6.8	0.49	0.16	0.06	4.42
pCHK1129-6	0.03	1.04	39.32	1.62	48.62	6.57	0.35	0.13	0.06	4.08
pCHK1129-7	0.03	1.23	32.2	2.05	55.64	6.38	0.42	0.16	0.05	1.57
pCHK1129-8	0.04	1.39	35.13	1.84	51.11	7.73	0.43	0.22	0.05	1.46
CHK100 Control	0.03	1.33	32.22	2.29	55.28	6.50	0.44	0.18	0.05	1.60

Example 35: Expressing *Volvulina compacta* (*VcomLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0309] *VcomLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1210 contained 5' and 3' homology arms to permit targeted integration of *VcomLPAAT2* into the genome and is shown in **Table 74**.

Table 74: Integrative sequences for the transformation of *P. moriformis* with pCHK1210 encoding *VcomLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
65	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgccctcttggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcggttgccactctccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgtgcagca gtccctctgcagcagatgagcgtgactttggccattcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgctccagatcgacagatagcgaccatgtgtgctgctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattctgtcaccatcgccatggagcaggctcacttagattccgate acccaacgcacatctcgctaatagtcattcgtgtctctcatcaatctcaagtgcgtgcatggatcttggttgacgatcgcggtatg ggtttgcgcgtggctgcagggtctgccaaggcaagctaaccagctcctctccccgacaatactctcgaggcaagccggtca cttgccttcagattgccaataaactcaattatggcctctgcatgccatccatgggtctgatgaatggtcacgctcgtgctcgtgaccgtt ccccagcctctggcgtccctgccccgccaccagccacgcgcgcgcagtcgtgccaaggctgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC

SEQ ID NO:	Nucleotide Sequence
	ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtctgcaggccttctgttctgtggccggttcgcccaagatcagcg ctccatgaacgaacgagacgtccgaccgccccctgggtgcacttcacccccacaagggtgtgatgaacgaccccaacggcctgtggtg cgcgcagaagggacgccaagtggcactgtacttcagtaaacccgaacgacaccgctctgggggacgccctgttctggggccacgc acgtccgacgacctgaaccactgggaggaccagcccatcgccatgcgccgaagcgcaacgactccggcgcttctccggctccatgg tgggtggactacaacaacacctccggcttcttaacgacaccatcgacccgcgccagcgctgcgtggccatctggacctacaacaccccg gagtcggaggagcagtaacatctctacagcctggacggcgctacaccttcaccgagtaccagaagaaccccgctgtggccgcaact ccaccagttccgcgacccgaaggttcttctggtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgcagatctactctccgacgacctgaagtctggaagtgtgagtcgcgttcgccaacgaggcgcttctcggtaccagtagcagtgccc cggcctgatcgagggtccccaccgagcaggacccagcaagtctactgggtgatgttcattccatcaaccccgccgccccggccggc ggctccttcaaccagtacttctgcgcgacgttcaacggcaccaccttcgagccctcgacaaccagtcccgctgtgtgacttcggcaag gactactacgcccctgcagaccttcttaacaccgacccgaacctacgggagcgccctgggcacgcgtgggctccaactgggagtactc cgccttcgtgcccaccaacccctggcgctcctccatgtccctcgtgcgaagtctccctcaacaccgagtaccaggecaacccggagac ggagctgatcaacctgaaggccgagccgatcctgaacatcagcaacgccggccccctggagccgggttcgccaccaacaccacgttgac gaaggecaacagctacaacgtcgacctgtccaacagcacccggcacccctggagttcgagctgtgtgtacgccgtcaacaccaccagac gatactcaagtcctgtgttcgcggacctctccctctggttcaaggccctggaggaccccgaggagtacctccgatgggttcgagtggtcc gcgtcctcttcttctgtgaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaccaaccgatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtcctactacaaggtgtacgcttctgtggaccagaacatcctgtgagctgtacttcaacgacg gcgacgtcgtgtccaccaacacctacttcatgaccaccgggaacgcctgggctccgtgaacatgacgacgggggtggacaacctgttc tacatcgacaagttccaggtgcgcgaggtcaagtgtTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGGCCGCGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcgcagcagctcggatagtatcacacactctggacgctgtgtgtgtggtgac tggttgcgcacaactgtgtccttgacctgtgaatatccctgccgttttatcaaacgcctcagtggtgtgtgtgtgtgtacgcgttttgcg agttgctagctgtgtgtgtatttgcgaataacacccccagcatcccttccctcgtttcatatcgcttgcattcccaaccgcaacttatctacgc tgctctgtatccctcagcgctgtctctgtctctgtcactgccccctgcacagccttggttgggttcgcctgtatttctctgtgtactgcaacc tgtaaacagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaaatggacctaggaaataacaatcaTCGCGG CTCAGCTACACGTCTTCTCCGATACCTTTCCCTCATTTGCATTTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCCGCTCGAATTGCTCGTGTGCGAGGCGGGGCACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCCTGACGACTGTAACCCCTGCCAGAAACAATTG AGCCTCAACAAGCCGAGTTGTGCAAAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC

SEQ ID NO:	Nucleotide Sequence
	<u>TTCCAGAAAGCATCCGGACGGGGGTAGCGAGGCTGTGTCTGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TCGACCCCCGTGGGCCGGGCCCCCGGACCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC</u> <u>A</u>ATGTGCTCATTAGCAAGGCCCTCTCCCTGCCAGCTTCCTGCTCTCCGTCTTCGTGT TCTACTGGTCGCTGCCGATCTTCGCCATCCCCCTACCGCATCCGGTTCGCCAACGTCGGC AAGCGGAACGACATGCTCGACTGGGCGAAGGCTCTGTTTCGCGTTTTTCCGCGTCACCGT CCTGAAGCAGGGGGAGCAGGGCCTCTACCGCGGTGGCCATGCCTGTACCTGTGCAAC CACCGCTCGTGGGCGGACTTCTTTGTGGACGTCTACCTGACGGAGGGCCGGGCGCGC TGATGTCCCGCTGGCTGGTGTTCCTGCTTCCCTGTGTTCTGCACCTCCTGCCTCATCC TGCGGGGCATCGTGTGTTCAAGCGCGGGACCATCCTCGACAAGGAGGCCTTTAACGC GTGGCTCGACGCCACGCTGCGGCGCTCCACCGTCCCCGGCCTGCTCATTACCCCGAG GGGCACCGCTCGCTGAAGCCGACCTCGCTGCCGCTGAAGCGGGGCATGCTGCACTTCG CTTACAGCCGACGGCTCCCCGTCCAACCTGATCGTGACCCGCGGCAAGGAGGACATCCT GTCGGAGAAGACGATGGCCATGAAGTACGGCCGACCCCTCGTGACCACGTACTCGAAG GTCCTGAAGCCCACGGACTACGACAGCTTCGACGCCTTCATGGGTGACGTCCAGACGA GCTGGGACGCGGGCTGGAAGGCCTCTACGAGGCCCCCAACAAGGGGTGCCCAACT GTCCATGGCGGGTGCCACGAGTACGACTACCGGGCGACCATGCGGTGGCAGCAACTG GCGATACCCCTCTTCTCGATCGTGTTCGCGGCCGTGCTGTACGGCAGTGCGTGGG GCCTGGGTGCCGTCTGGCTGCGGCTGGCCCCCTCGGCCAGCAAGTGGTCTGGTCTCT GGCGGCCACCTCCTGGCTGGGCGCCAGCCTGATCCGGTCTGCCGCTAG^{gtaccacgcccgcg} ^{cggcgacactgacctgttctctc}gagggcgctgttctgccttgcgaacaagcccctggagcatcgtgcatatcgtctctgcccgcg ^{cgcgcggttctc}gcccctcgcggcgccccggcgcgggggcgccattgaaattgttgcaaacccacctgacagattgaggcccaggca ^{ggaaggcgttgatg}gaggtacaggagtaagtaactgaaagtgtttatgataactaacaacaaagggtcgtttctgcccagcgaatgaca ^{gaacaagattccacatttccgt}tagaggttgcctcgaatgtgagcggcgggcgccgacccgacaaaaccccttacgacgtggtgaaga ^{aaaacgtg}cgggcactgtccctgtagcctgaagaccagcaggagacgatcgaagcatcacagcacggccgccccgctctcgaaca ^{gagcgcgcgcgccgc}gatacctgcaggacagcgccatgccacgcccttfgatggcttcaagtacgattacgggtgttgattgtgtg ^{ttt}gttcgtagtgtcatggtttagaataatacattgatttctgtcacggcaatctcggttgcgcaggttcaacccatttcgga ^{gtctcaggtcagcgcg}caatgaccagcgtacttcaaggacttgacgacaacgccgaggtgagctatgtttaggacttgattgga ^{aattgtcgtcagc}catatttcgctccgacagcaccaagcaaatgtcaagtgcgttcgatttgcgtccgaggtgatgtgtg ^{gatcgtcggcgccgatccg}ggtctgtctgcgttacgagctgaccaagcaccctgacgtccgggtacgcgagctgagattcgat ^{tagacataaattgaagattaa}ccccgtagaaaaattgatggtcggaactgtctcgattgcaagaaattgatcgtctccactcc ^{gcaggtcgccatcatc}gagcagggcggttctcccggcgggcgccctggctggggggacagctgttctcgccatgtgtgtacgtag ^{aaggatgaattcagctggtt}tcgtgcacagctgttctgtcatgattgttgcagactattgtgaatgttttagatttcttaggatgcat ^{gattt}gtctgcatgcgact

[0310] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:*VcomLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *VcomLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *VcomLPAAT2*. The initiator ATG and terminator TAG codons of the *VcomLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0311] **Table 75** shows the fatty acid/TAG profiles of primary transformants of CHK22 transformed with pCHK1210. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 75: Screen of primary transformants of CHK22 with pCHK1210.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1210-1	0.04	1.34	27.76	2.47	57.59	8.42	0.51	0.22	0.00	4.25
pCHK1210-2	0.04	1.34	27.91	2.78	57.65	7.96	0.51	0.25	0.00	3.93
pCHK1210-3	0.03	1.30	27.68	2.63	58.35	7.58	0.48	0.23	0.00	4.24
pCHK1210-4	0.04	1.29	27.75	2.60	58.19	7.69	0.49	0.23	0.00	3.75
pCHK1210-5	0.04	1.37	28.03	2.42	57.05	8.60	0.49	0.23	0.00	4.39
pCHK1210-6	0.04	1.34	28.03	2.75	57.66	7.72	0.50	0.24	0.00	3.69
pCHK1210-7	0.04	1.32	27.80	2.66	57.78	7.91	0.51	0.23	0.00	3.97
pCHK1210-8	0.04	1.34	28.09	2.83	57.40	7.87	0.51	0.25	0.00	3.94
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 36: Expressing *Vitreochlamys* sp. CL-2021 (*VitrLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0312] *VitrLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1211 contained 5' and 3' homology arms to permit targeted integration of *VitrLPAAT2* into the genome and is shown in **Table 76**.

Table 76: Integrative sequences for the transformation of *P. moriformis* with pCHK1211 encoding *VitrLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
66	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgcctccttggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgttgcactctgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgtcgagca gtccctctgcagcagatgagcgtgactttggcatttcacgcactcgagtgtacacaatccattttcttaaagcaaatgactgtgatt gaccagatactgtaacgtgatttcgtctccagatcgacagatagcgaccatgttgcgtcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattctgtcaccatcgccatggagcagggtccacttagattcccgate accacgcacatctcgctaatagtcattctgtctgtctgatcaatctcaagtgcgtgcatggatcttgggtgacgatcgcggtatg ggtttgcgcgtggctgcagggtctgccaaggcaagtaaccagctcctctccccgacaatactctcgaggcaaaagccggtca cttgccttccagattgccaataaactcaattatggcctctgtcatccatccatgggtctgatgaatgggtcacgctcgtgtcctgaccgtt ccccagcctctggcgtccccctgccccgccaccagccccacgcgcggcagtcgctgccaaggtgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCCTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACTGTACAATGctgtcgcagggccttctgttctgtctgcccggcttcgccccaagatcagcg cctccatgacgaacgagacgtccgacggccccctgtgcaactcaccaccaaaagggtgatgaacgaccccaacggcctgtgta cgacgagaaggacgccaagtggcactgtactccagtacaacccgaacgacaccgtctggggacgcccttgttggggccacgcc acgtccgacgacctgaccaactgggaggaccagccccatcgccatcgccccgaagcgcaacgactccggccttctccggctccatgg tggtggaactacaacaacacctcggttcttcaacgacaccatcgacccgcgcagcgtcgtggccatctggacctacaacacccc gagtcgaggagcagtagatctctacagcctggacggcggtacaccttaccgagtagcagaagaaccccgctggtggcccaact ccaccagttccgcgacccgaaggtcttctgtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactcctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggttctcggctaccagtagagtccc cggcctgatcgaggtccccaccgagcaggacccccagcaagtcctactgggtgatgttcattccatcaaccccgcgccccggccggc ggctccttcaaccagtacttcgtcgagcttcaacggcacccacttcgaggccttcgacaacagtcgccggtgtggacttcggcaag gactactacgccctgacaccttcttcaacaccgacccgacctacgggagcgccttggcatcgctggccctccaaactgggagtactc cgcttcgtgcccaccaacccctggcgctcctccatgtccctgtgcgaagtctccctcaacaccgagtagcaggccaacccggagac ggagctgatcaacctgaagccgagccgactctgaacatcagcaacgcccggccctggagccggttcgccaccaacaccacgttgac gaagggccaacagctacaacgtcagctgtccaacagcaccggcacccctggaggttcgagctggtgtacgccgtcaacaccaccagac gatctcaagtcggtgttcgagacctctcctctgttcaaggccctggaggaacccgaggagtacctccgcatgggttcgaggtgac

SEQ ID NO:	Nucleotide Sequence
	g c g t c c t c c t t t c t c t g g a c c g c g g g a a c a g c a a g g t g a a g t c g t g a a g g a g a a c c c t a c t t c a c c a a c c g c a t g a g c g t g a a c a a c c a g c c c t t a a g a g c g a g a a c g a c c t g t c t a c t a c a a g g t g t a c g g c t t g c t g g a c c a g a a c a t c c t g g a g c t g t a c t t c a a c g a c g g c g a c g t c g t g t c c a c c a a c a c c t a c t t c a t g a c c a c c g g g a a c g c c c t g g g t c c g t g a a c a t g a c g a c g g g g t g g a c a a c c t g t t c t a c a t c g a c a a g t t c c a g g t g c g c g a g g t c a a g t g a c t T A A G A C G C C C G C G C G G C G C A C C T G A C C T G T T C T C T C G A G G G C G C C T G T T C T G C C T T G C G A A A C A A G C C C C T G G A G C A T G C G T G C A T G A T C G T C T C T G G C G C C C C G C C G C G C G G T T T G T C G C C C T C G C G G G C G C C G C G G C C G C G G G G C G C A T T G A A A T T G T T G C A A A C C C C A C C T G A C A G A T T G A G G G C C C A G G C A G G A A G G C G T T G A G A T G G A G G T A C A G G A G T C A A G T A A C T G A A A G T T T T A T G A T A A C T A A C A A C A A A G G G T C G T T T C T G G C C A G C G A A T G A C A A G A A C A A G A T T C C A C A T T T C C G T G T A G A G G C T T G C C A T C G A A T G T G A G C G G G C G G G C C G C G G A C C C G A C A A A A C C C T T A C G A C G T G G T A A G A A A A C G T G G C G G G C A C T G T C C C T G T A G C C T G A A G A C C A G C A G G A G A C G A T C G G A A G C A T C A C A G C A C A A C C G G T g c a g c a g c a g c t c g g a t a g t a t c g a c a c a c t t g g a c g t g t g t g t g a c t g t t c c g c c a c a c t t g t g c t t g a c c t g t g a a t a c c c t g c c g t t t a t c a a c a g c c t c a g t g t g t t g a c t t g t g t a c g c g t t t g c g a g t t g t a g t g c t t g t g c t a t t t g c a a t a c c a c c c c a g a t c c c t t c c t g t t c a t a c g t t g c a t c c a a c c g c a a c t a t a c g c t g t c t g t a t c c c t c a g e g t g e t c c t g c t c t g t c a c t g c c c t c g c a c a g e c t t g g t t g g g c t c c g c t g t a t t c t c t g t a c t g c a a c c t g t a a c c a g c a c t g c a a t g t g a t g c a c g g g a a g t a g t g g g a t g g g a a c a c a a t g g a c c t a g g a a t a a c a a t c T T C G C C G C T C A G C C T A C A C G T C T T C T C C G A T A C C T T T C C C T C A T T G C A T T T A T G C C A G A C T G G G T C C C A G C C T G G G T G G G T G C T C C C G C T C G A T T G C T C G T G T C G G A G G C G G G G C A C C C C G C T C T C T C T A T T T A T C A C T G C C T C T C C C C G A C C A A C C C T G A C G A C T G T A A C C C T G C C A G A A A C A A T T C A G C C T C A A C A A G C C G A G T T G T G C A C A A G G G A G A C T A A T T T T T A G A C G G G A A G C A A C C C G C T T C C A G A A G C A T C C G G A C G G G G T A G C G A G G C T G T G T C G A G C G C C G T G G G G A T C T G G C C G A T G A G G T G C C C G A A A C C C G T G T A C A G C T C A G C G G C T G G G A T C A T C G A C C C C G G G A T C A T C G A C C C C G T G G G C C G G G C C C C G G A C C C T A T A A C T A A A A G C C G A C G C C A G C G A A A A C C A C A A A C A T T T A C T C C T T A A T C C T C G C T C C T C C T T C A T A T A C A A C C A C A A G T A A T C A A C T C A C C A A T G T C G C T G A T C A G C A A G G C C T C T T C C C T G C C G A G C T T C C T G T T T T C G G T G T T C G T C T T C T A C T G G T C C C T G C C A A T C T T T G C G G T C C C G T A C C G T A T C C G G T T T G C T A A C A T G G G G A A G C G T A A C G A C A T G C T G G A C T G G G C T A A G G C G C T G G T G G C C T T T T C C G C G T C A C C G T C C T G A A G C A G G G C G A G C A G G G C C T G T A C C G G G C G G G A C G T G C C T C T A C C T G T G C A A C C A C C G A A G C T G G G C C G A C T T T T C A T C G A C G T C T A C C T G A C G G A G G G C C G T G C G G C C C T G A T G T C G C G G T G G C T C G T G T T T T C G T C T T C C C T G T C T T T T G C A C G A G C T G C C T C A T C C T C C G A G G C A T C G T C C T G T T A A G C G G G G C A C G A T C C T G G A C A A G G A G G C C T T C A A C G C C T G G C T C G A C G C C A C G C T C C G A C G C T C G A T C G T G C C T G G C C T G G T G G T C T A C C C C G A G G G G C A T C G T C C C T C A A G C C C A C C T C G C T C C C C C T G A A G C G A G G C A T G C T G C A C T T C G C G T T T G G C C G T C A G C T C C C G G T G C A A C T G A T C G T G A C G C G C G G A A A G G A G G A C G T G C T G T C C G A G A A G A C C A T G G C C A T G A A G T A C G G C C G C A C G C T G G T G A C G A G C T A C T C T A A G G T G C T G A A G C C C T C T G A C T A C G C C A G C T T C G A C G C C T T C A T G A C G G A C A T C C A G A C G T G C T G G G A C T C T T G C T G G C G T G C C G C T T A C G A G G C C C G A C C A A G G G C G T C C C C A G C T

SEQ ID NO:	Nucleotide Sequence
	<i>CTCCATGGTCGGCGCCACGAGTACGACTACCCCGGACCATTGGTGGCAGCAACTCTGGATCACCTGGCCTCCATCCTGATCTTCGCTGGCGTGCTCTACGGCACCTGGCGCGGCCTGGCTCCCTCCTGGCTGCCGCGACCACGAGCGGCGCGCTGGGCCCACAGGTCCTGGTGACCGTGGCCGCGGCCTGGGCGGGCTCCTCGCTCCTGCGGTCGCTGCTGTGA</i><u>gtaccacgcccgcgcgcgcacctgacctgttctctcagggcgccgtgtctgccttgcgaaacaagccctggagcatcgctgcatgatcgtctctgcgccccgcgcggtttgtcgcctcgcggcgccccggccgcggggcgccattgaaattgttgcaaacccacctgacagattgagggccaggcaggaaggcggtgagatggaggtacaggagtcagtaactgaaagttttatgataactaacaacaagggtcgtttctgccagcgaa</u> <u>tgacaagaacaagattccacatttcgttagagccttgccatggaatgtgagcggcgggccgcggaccgacaaaacccttacgacgtgtaagaaaaacgtggcgggcactgtccctgtagcctgaagaccagcaggagacgatcggaagcatcacagcacagccggccccgcgtctgaacagagcgcggcgccgcgcgcgatacctgcaggacagcgccatgccacgccctttgatggcttcaagtacgattacgggtgtggattgtgtgtttgtcgtagtgtgcatggtttagaataatacacttgatttctgtcacggcaatctcggttgcgcaggttcaaccccat</u> <u>ttcggagttcaggtcagcgcgcaatgaccagccgctacttcaaggacttgcacgacaacgccagggtgagctatgttaggacttgattgaaattgtcgtcagcgcataatcgcgctccgcgacagcacccaagcaaaatgtcaagtgcgttccgatttgcgtccgaggtcgatgtgtgatcgtggcgccgatcccggtctgtcctgcgcttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattagacataaattgaagattaaacccgtagaaaaattgatggtcgcaaaactgtctcgattgcaagaaattgatcgtctc</u> <u>cactccgcaggtgccatcatcgagcagggcggtgtctccggcgggcgccctggctggggggacagctgttctcgcccatgtgtgtagcgtagaaggatgaatttcagctggtttcgttgcacagctgtttgtgcatgattgtttcagactattgtgaatgttttagatttcttagg</u> <u>atgcatgattgtctgcatcgact</u>

[0313] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:*VitrLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *VitrLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *VitrLPAAT2*. The initiator ATG and terminator TGA codons of the *VitrLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0314] **Table 77** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1211. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in the lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 77: Screen of primary transformants of CHK22 with pCHK1211.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1211-1	0.04	1.34	27.64	2.85	58.00	7.77	0.48	0.25	0.00	4.06
pCHK1211-2	0.04	1.36	28.07	2.68	57.65	7.88	0.49	0.24	0.00	4.13
pCHK1211-3	0.04	1.35	27.74	2.58	57.77	8.18	0.50	0.23	0.00	4.28
pCHK1211-4	0.04	1.37	27.89	2.71	57.61	8.03	0.52	0.24	0.00	3.99
pCHK1211-5	0.04	1.33	27.71	2.65	58.04	7.82	0.50	0.23	0.00	4.16
pCHK1211-6	0.04	1.60	31.43	2.20	54.50	7.52	0.50	0.21	0.00	4.07
pCHK1211-7	0.04	1.36	27.57	2.79	58.16	7.68	0.48	0.24	0.00	3.94
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 37: Expressing *Colemanosphaera charkowiensis* (*CchaLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0315] *CchaLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1212 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 78**.

Table 78: Integrative sequences for the transformation of *P. moriformis* with pCHK1212 encoding *CchaLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
67	ccctcaactgcgacgtgggaaccttctccgggcaggcgatgtgcgtgggttgccctctggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgctgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgtccagatgcacagatagcgaccatgttgcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgaacagatggcgacacttggtacaattcctgtcaccatcggcgatggagcagggtccacttagattccgac accacgcacatctcgtaatagtcattcgttctgtcttcgatcaatcaagtgcgtgtgcattggttgacgatgcggtatg ggtttgcgcgctggctgcagggtctgccaaggcaagtaaccagctcctctcccgacaatactctgcgaggcaagccggtca cttgcctccagattccaataaactcaattatggcctctgcatgccatccatgggtctgatgaatggtcacgctcgtgtcctgaccgtt

SEQ ID NO:	Nucleotide Sequence
	<u>ccccagcctctggcgctccccctccccgccaccagcccaacgcgcggcagtcgctgccaaggtgtctcgga</u><u>CTTCTTGC</u> <u>GCTATGACACTTCCAGCAAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC</u> <u>ATGCAACACCGATGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT</u> <u>CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC</u> <u>ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCAC'TTCTACACA</u> <u>GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA</u> <u>GTCACAACCCGCAAACCTGTACA</u><u>ATG</u>ctgctgcaggccttctgttctgtgtggcgcttcgcccaagatcagcg cctcatgacgaacgagacgtccgaccgccccctgggtgcacttcacccccacaagggttgatgaacgaccccaacggcctgtggtg cgacgagaaggacgccaagtggcacctgttacttcagtacaaccggaacgacaccgctgtggggagcgccctgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgcccggaagcgcaacgactccggcgcccttctccggtccatgg tggtggactacaacaacacctccggcttcttaacgacaccatcgaccgcgccagcgctgcgtggccatctggacctacaacacccg gagtcggaggagcagtacatctctacagcctggacggcggtacaccttcaccgagttaccagaagaaccccgctgtgtgcccaact ccaccagttccgcgacccgaagggttcttggtacgagccctccagaagtggatcatgaccgggcccaagtccaggactacaagat cgagatctactctccgacgacctgaagtctggaagtggagtcgcggttcgccaacgagggcttctcgtggtaccagttacgagtccc cggcctgategaggtccccaccgagcaggacccccagcaagttctactgggtgatgttcatctccatcaaccccgccgccccggcgcc ggctcttcaaccagttacttctgcgcgagcttcaacggcaccaccttcgaggccttcgacaaccagtcgccgtgtgtgacttcggcaag gactactacgccctgcagaccttcttaacaccgacccgaactacgggagcgccctgggcatcgctgggacctcaactgggagtactc cgcttcgtgtcccaccaacccctggcgctctccatgtccctcgtgcgaagttctccctcaacaccgagtaccagggcaacccggagac ggagctgatcaacctgaaggccgagccgatctgaacatcagcaacgccggccccctggagccgggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcacctggaggttcgagctggtgtacgccgtcaacaccaccagac gatctccaagtccgtgttcgcggacctctccctctggttcaaggccctggaggaaccccgaggagtacctccgcatgggcttcgaggtgtcc gcgtctctcttctctggaccgcggaacagcaaggtgaagtctgtgaaggagaacccctacttcaccaaccgcatgagcgtgaacaa ccagccctcaagagcgaagacgacctgtctactacaaggtgtacggttctgtggaccagaacatctgtgagctgtacttcaacgacg gcgacgtcgtgtccaccaacacctacttcatgaccaccgggaacgccccgggctccgtgaacatgacgacgggggtggacaacctgttc tatatcgacaagttccaggtgcgcgaggtcaagtgaactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGT'TCTGCCTTGCGAAACAAGCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCCGCGCGGTTTGTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCGCGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcgtagatctgcacacactctggacgctgtgtgtatggac tgttgcgcgcaactgtgtgcttgacctgtgaatatccctgcgctttatcaaacagcctcagtggtgtttgactgtgtgtacgcgcttttgcg agttgctagctgtgtgtctatttgcgaataccacccccagcatcccttccctcgtttcatatcgcttgcatcccaaccgcaacttatctacgc tgtctctctatccctcagcgctgtctgtctctgtctactgccccctgcacagccttggttgggtccgcctgtatttctctgtactgcaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatcaTTCGCCG

SEQ ID NO:	Nucleotide Sequence
	<u>CTCAGCCTACACGTCTTCTCCGATACCTTTCCCTCATTGCATTTTATGCCAGACTGGGTCCC</u> <u>AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGCACCCCCGCTCTC</u> <u>TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAAACCCTGCCAGAAACAATTC</u> <u>AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC</u> <u>TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGCGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TGCACCCCGTGGGCGGGCCCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC</u> <u>A</u>ATGTCCATCGTCACCAAGGTGCTGAACCTCCCCTCGTTCCTGTTACGCGTGTTCGTCT TTTACTGGTCCCTGCCCCCTGTTTGCATCCTGTACCGCATCCGCTTCGCCAACGTGGGC AAGCGCAACGACATGCTGGACTGGGCGAAGACGCTCGTCGGCTTTTTCCACGTGACCA TCCTGAAGCAGGGCGAGCTGTCTCTTACCAGGACGGGCGGTGCCTGTACCTGTGCAA CCACCGCTCCTGGGCCGACTTCTTTATCGACACCTACCTGACGGAGGGTCGCGCTGCCC TGATGAGCCGCTGGCTGGTGTACTTCGTGTTCCCGGTCTTCTGCACGTCCGTGATTATC CTGCGCGGCATCGTGCTGTTCAAGCGGGGTCTATCGCGGACAAGGAGGCCTTCAACG CGTGGCTGGACGCCACGCTGCGTAAGTCCATTGTCCCCGCGCTCCTGGTGTACCCGGA GGGCCACCGCTCGCTGCGCCCCACGTCTCTCCCTCTGAAGCGCGGCATGCTGCACTAC GCCTACAGCCGCCGACTCCCTGTGCAGCTCATCGTCACGTGCGGCAAGGAGGCCGTCA TGAGCGAGAAGACCATGAGCGTCCGCTTCGGCCGACCCCTCGTCACCACGTACTCCAA GGTGCTGCGCCCCGCGGACTACCCACGTTTGACGCCTTCTTTCCGACATCCAAGCTA CCTGGGACGGCTGCTGGGACGCCGTTACAAGCAGCCGTCTGCGGGTGCGCCCCAGCT CCGCCTGGTGGACCCGCATGGCTACAACCTACCCGGCCCCGCATGTGGTGGCTCCAACGTG GGCGTGACCTCCTGTGATCGGGGTGATGGCCGCTGTGATCGCCGCGAGCTGGTGGG GTCTGGGCGCTCTCCTGGCCGCGACGGGCCCCGGCTAAGCAGGTGGTCTGGGCGTCGT GGTCTCGCTGCTCGGCGCGAGCCTCCTGCGATCGTGCTGTGA^{gtaccacgcccgcgcggcgacc} ^{tgacctgttctc}gagggcgctgttctgccttgcaaaagccctggagcatgctgcatgctgtctgtgcgccccccgcgcggtt ^{gtcgccctcgcggcgccccggccgcggggcg}cattgaaattgttgcaaacccacctgacagattgaggccccaggcagggaaggcgtt ^{gagatggaggtacagagtc}aagtaactgaaagttttatgataactaacaacaaagggtcttctgcccagcgaatgacaagaacaagattc ^{cacatttcctgtagaggttgc}catcgaatgtgagcggcgggccgcggacccgacaaaaccttacgacgtgtaagaaaaacgtggcg ^{ggcactgtccctgtagcctgaagaccagcaggagacgac}ggaagcatcacagcacaggccggcccgctctcgaacagagcgcggcg ^{ggcgcatcgatacctgcaggacagcgccatgccacgcctt}gatggcttcaagtacgattacgggtgtggattgtgtgttgtgtgtag ^{tgtgcatggtttagaataatacacttgatttctt}gtcagcgcaatctcggtgtccgcaggttcaacccatttcggagtctcaggta ^{gccgcgcaatgaccagcgctacttcaaggacttgcacgacaac}gaggtgagctatgttaggacttgattggaaattgtcgtc ^{acgcataatcgctcgcgacagcacccaagcaaatgtaagtgcgttcg}atttgcgtccgaggtcgatgtgtgatcgtcggc ^{gccggatccg}cggtgtcctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattagacataa ^{attgaagattaaacccgtagaaaaattg}atggtcgcgaaactgtgctcgattgcaagaaattgatcgtctccactccgcaggtcgc ^{catcatcgagcagggcggtgtc}ccggcgggcgccctggctggggggacagctgttctcgccatgtgtgtacgtagaaggatgaa

SEQ ID NO:	Nucleotide Sequence
	tttcagctgggtttcggtgcacagctgtttgtgcatgattgtttcagactattgtgaatgttttagatttcttaggatgcatgattgtctgcatgcgact

[0316] The construct can be written as

5'Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*CchaLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *CchaLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *CchaLPAAT2*. The initiator ATG and terminator TGA codons of the *CchaLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0317] **Table 79** shows the fatty acid/TAG profiles of eight primary transformants of CHK22 transformed with pCHK1212. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 79: Screen of primary transformants of CHK22 with pCHK1212.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1212-1	0.04	1.32	27.29	2.88	58.69	7.56	0.54	0.24	0.03	2.4
pCHK1212-2	0.03	1.24	27.36	2.92	59.47	6.87	0.50	0.23	0.00	2.2
pCHK1212-3	0.03	1.22	27.50	2.91	59.53	6.70	0.49	0.23	0.00	1.8
pCHK1212-4	0.03	1.25	27.81	2.75	58.58	7.43	0.49	0.21	0.03	2.5
pCHK1212-5	0.03	1.33	27.56	2.81	58.47	7.62	0.51	0.22	0.00	2.6

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1212-6	0.03	1.30	27.79	2.79	58.61	7.34	0.51	0.22	0.00	2.2
pCHK1212-7	0.03	1.27	27.96	2.68	58.71	7.18	0.51	0.21	0.00	2.3
pCHK1212-8	0.04	1.34	27.76	2.94	58.84	7.00	0.50	0.22	0.00	2.1
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 38: Expressing *Pleodorina japonica* (*PjapLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0318] *PjapLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct, pCHK1213, contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 80**.

Table 80: Integrative sequences for the transformation of *P. moriformis* with pCHK1213 encoding *PjapLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
68	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgccctcttggcagcgcttacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgttgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgctgcagca gtccctctgcagcagatgacgtgacatttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgtccagatgcacagatagcgacctgtgtcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattcctgtcacccatcgcatggagcaggctccacttagattcccgatc acccacgcacatctcgtaatagtcattcgttctgtcttcgatcaatcgaagtgcgtgcattggttgacgatgcggtatg ggtttgcgcgtggctgcagggtctgccaaggaagtaaccagctcctctcccgcacaatactctgcaggcaaaagcggtca cttgccttcagattgccaataaactcaattatggcctctgtcatgcatccatgggtctgatgaatggtcacgctcgtgtcctgaccgtt ccccagcctctggcgctcccctgccccgccaccagccacgcgcggcgagtcgctgccaaggctgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGAAACTGTACAATGctgtgcaggccttctgttctgtgtggccgcttcgccaagatcagcg cctccatgacgaacgagacgtccgacgccccctgggtgcacttcccccaacaagggtggtgaacgaccccaacggcctgtgga cgacgagaaggacgccaagtggcacctgtacttcagatcaacccgaacgacaccgtctggggacgacctgttctggggccacgc acgtccgacgacctgaccaactgggaggaccagcccatcgccatgccccgaagcgcaacgactccggcgcccttcgggtccatgg tggtggactacaacaacacctcggcttctcaacgacaccatcgacccgcgcagcgctgctggccatctggacctacaacaccccg

SEQ ID NO:	Nucleotide Sequence
	gagtcgaggagcagtagcatctctacagcctggacggcggtacaccttcaccgagtagcagaagaacccccgtgctggccccaact ccaccaggtccgcgacccgaaggctcttggtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagctctggaagctggagtcgcgttcgccaacgagggcttctcggtaccagtagcagtgccc cggcctgatcgaggtccccaccgagcaggaccccaagaagtcctactgggtgatgttcacatccatcaacccccggcggccggc ggctcctcaaccagtagcttctgctggcagcttcaacggcacccttcgaggecttcgacaaccagtagccgcgtgggtgacttcgcaag gactactagccctgcagaccttctcaacacccgacccgacctagggagcgccctgggcatcgctgggctccaactgggagtactc cgcttcgtgcccaccaacccccggcgtcctcatgtccctgctgcgaagttctcccaacaccagtagtaggccaacccggagac ggagctgatcaacctgaaggccgagccgacccgaacatcagcaacgcggccctggagccgggtcggccaacacccaggttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcaccctggagttcgagctgggtgacgccgaacacccacccagac gatctcaagtcggttcgagacctctccctggttaaggccctggagaccccgaggagtacctccgcatgggcttcgaggtgtcc ggctcctcttctctgagccggggaacagcaaggtgaagttcgtgaaggagaacccctacttcaccaaccgcatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtctactacaaggtgtacggcttctgtaggaccagaacatcctggagctgtactcaacgacg ggcagctgctgtccaccaacacacttcatgaccaaccgggaacgccctgggctccgtgaacatgacgacgggggtgacaacctgttc tatatcgacaagtccaggtgcgcgaggtaagtgactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA GGGTCTGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCCCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcgatagatcgacacactctggacgctggtgctgtgatggac tgttgcggccacactgtgctgctgacgtgtgaatccctgcgctttatcaaacagccctagtggtttgatcttgtgtgtacgcgctttgcg agttgctagctgcttgtctatttgcaatacaccacccagcacccttccctcgttccatctgctgcatcccaaccgcaactatctacgc tgtctgtatccctcagcgctgctctgctcgtcactgcccctgcacagccttggttgggtccgctgtattctctggtactgcaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacntaggaataacaatcaTTCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCCTGACGACTGTAAACCTGCCAGAAACAATTG AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA TCGACCCCGTGGGCGGGCCCCCGGACCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCTTAATCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC AATGTCCGTGATCTACAAGGCTCTGTGCTGCCATCCTTCTGTCTCTGTGTTCTGTGTTCTGTGTT CTACTGGTCTCTGCCCCGTTCGCGTCTGTCTACCGCATCCGGTTTGCGAACCTCGGCA AGCGCAACGACATGCTGGACTGGGCGAAGGCCCTCGTGGCCTTCTTTCGCGTCACCGT

SEQ ID NO:	Nucleotide Sequence
	<i>GCTCAAGCAAGGCGAGATGTCGCTCTACCGCGGAGGCCCTTGTCTCTACCTGTGCAACC</i> <i>ATCGGTCGTGGGCTGACTTCTTTATCGACACGTACCTGACCGAGGGCCGCGGGCCCT</i> <i>GATGAGCCGCTGGCTGGTCTACTTCGTGTTTCCGGTGTTTGTACCAGCGTGCTGATCC</i> <i>TGCGGGGCATCGTCCTGTTTAAGCGGGGTACATCGTGGACAAGGAGGCGTTCAACGC</i> <i>GTGGCTCGACGCCACCCTGTTCAAGTCTACGGTGCCGGGCCTCCTGGTGTACCCCGAG</i> <i>GGCCACCGCTCCCTCAAGCCCGCTCCCTGCCCCCTGAAGCGTGGCATGTCCACTTTGC</i> <i>TTTTTCCGCAAGCTGCCCGTCCAGGTCATTTGACCCGCGGCAAGGAGGAGGTCCTGT</i> <i>GCGAGAAGACGCTGTCCGCGCGGTGGGGCCGCACGCTGGTGGTACCTTCTCCAAGGT</i> <i>GATCAAGCCGTCCGAGTACGACTCGTTCGACGCGTTTTTCAACGACGTGCAATCTACCT</i> <i>GGGACGCGTGCTGGCAGCTCTCCTACCACAGCCAACGGCGGGGCGTCCCGGAGCTGTC</i> <i>CATGAAGGACGCCCACGAGTACGACTACCCCGCTCTATGTGGTGGCTGCAACTGGGT</i> <i>ATCACCTCCTGTCCTTTATTATCTGGCCGCGGTGCTGTGGGGCTCTTGGGTGCTGT</i> <i>GCGCGCGGGTTTCGCCACGCTCGGCAGCGTCCAACAGGCCGTGGTCCTGGGCACCGCG</i> <i>GGGTGGATCGGCGCCTCGATGGTGTGCGGCTTCCTCTGA</i> <u>gtaccacgccccgcggcgccactgacct</u> <u>gttctctcgaggcgccctgttctgcttgcgaacaagccccgtgagcatgctgcatgctctctggcgccccgcggcggttgcgc</u> <u>cctcgcgggcgccccgcccgcggggcgccattgaaattgttgcaaacccacctgacagattgaggccccaggcaggaaggcggttgagat</u> <u>ggaggtacaggagtcgaagtaactgaaagtattatgataactaacaacaagaaggtcgttctgcccagcgaatgacaagaacaagattccacat</u> <u>ttcgtgtagaggcttgcctcgaatgtgagcgggcgccgcgggacccgacaaacccttacgacgtgtaagaaaaacgtggcgggca</u> <u>ctgtccctgtagcctgaagaccagcaggagacgatcggaagcatcacagcacaggccggccccgcgtctcgaacagagcgcgcgccg</u> <u>catcgatactgcaggacagcgccatgccagccctttagtggttcaagtacgattacgggtgttgattgtgtttgttgctagtg</u> <u>catggtttagaataatacattgatttctgtcacggcaatctcggtgttcgcaggttcaacccatttcggagctcaggtcagccg</u> <u>cgcaatgaccagcgctacttcaaggactgtcacgacaacgcccaggtgagctatgtttaggacttgattggaattgtcgtcgacgc</u> <u>atattcgcgctcgcgacagcaccaagcaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgtgtgtgatcgctcgccg</u> <u>gatcgccgggtgtgtctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattagacataaattga</u> <u>agattaaacccgtagaaaaattgatgtgcgaaactgtgtcgattgcaagaaattgatgtctccactccgagggtcgccatca</u> <u>tcgagcagggggtgtctccggcgccgctggtgggggacagctgttctcgccatgtgtgtacgtagaaggatgaatttca</u> <u>gtggttttctgtcacagctgtttgtcatgattgtttcagactattgttgaatgttttagatttcttaggatgcatgattgtctgcatgc</u> <u>gact</u>

[0319] The construct can be written as
5’Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*PjapLPAAT2*:PmPGH::3’Thia4.
Proceeding in the 5’ to 3’ direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding

region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *PjapLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. A PmG3PDH promoter from *P. moriformis*, indicated by the boxed italicized text, drives the expression of the *PjapLPAAT2*. The initiator ATG and terminator TGA codons of the *PjapLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0320] Table 81 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1213. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 81: Screen of primary transformants of CHK22 with pCHK1213.

Sample Name	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1213-1	0.04	1.32	28.01	2.75	58.23	7.17	0.50	0.25	0.00	1.87
pCHK1213-2	0.04	1.28	27.71	2.78	58.77	6.96	0.49	0.25	0.00	1.89
pCHK1213-3	0.04	1.35	28.46	2.96	57.69	7.00	0.49	0.27	0.00	1.55
pCHK1213-4	0.04	1.44	28.22	2.84	57.69	7.37	0.47	0.26	0.00	2.04
pCHK1213-5	0.04	1.50	27.69	2.33	59.75	6.46	0.32	0.18	0.00	1.85
pCHK1213-6	0.03	1.30	28.21	2.75	58.45	6.92	0.48	0.25	0.00	1.90
pCHK1213-7	0.06	1.67	19.16	4.74	61.76	9.66	0.88	0.52	0.00	1.50
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 39: Expressing *Volvulina boldii* (*VbolLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0321] *VbolLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct, pCHK1214, contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 82**.

Table 82: Integrative sequences for the transformation of *P. moriformis* with pCHK1214 encoding *VbolLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
69	ccctcaactgcgacgctgggaacctctccgggcaggcgatgtgcgtgggtttgcctccttggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgctgcagea gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttcttaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgtccagatcgacagatagcgacatgttgcgtgcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattcctgtcacccatcgcatggagcaggctccacttagattcccgate acccacgcacatctcgtaaatagtcattcgttctgtcttcgatcaatctcaagtgagtgatgcatggttgggtgacgatgcggtatg ggtttgcgcgctggctgcagggtctgccaaggcaagctaaccagctcctctcccgcacaatactctcgaggcaaaagccggtca cttgccttcagattgccaataaactcaatfatggcctctgcatgccatccatgggtctgatgaatggtcacgctcgtgtcctgacgggtt ccccagccttggcgctcccctgccccgccaccagccacgcgcggcgagtcgctgccaaggtgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCC GGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGAAACTGTACAATGctgtgcagccttctgttctgtgtgcccgttcgcccaagatcagcg cctccatgacgaacgagacgtccgacgccccctgggtgcacttcaccccccaacaaggctggatgaacgacccccacggcctgtggtgta cgacgagaaggacgccaagtggcacctgtacttcagtaaacccgaacgacaccgtctgggggacgcccttgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcccttctccggtccatgg tggtggactacaacaacacctccggttcttaacgacaccatcgacccgcgcagcgctgcgtggccatctggacctacaacacccccg gagtcgaggagcagatcatctctacagcctggacggcggtacaccttcaccgagtaccagaagaacccccgtgtggccgccaact ccaccagttccgcgacccgaaggcttctgtgtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagtcctggaagctggagtcgcgttcgcaacgagggttctcctggctaccagtagagtgccc cggcctgatcgaggtccccaccgagcaggacccagcaagtctactgggtgatgttcattccatcaacccccggcgccccggcgcc ggctccttcaaccagtaacttcgtcggcagcttcaacggcacccattcagagccttcgacaacagatcccgcgtgtggacttcgcaag gactactacgccctcgagacttcttaacacccgacccgacctacgggagcgccctgggcatcgctgggctccaaactgggagtactc cgcttctgtccccaccaacccctggcgctctccatgtccctcgtgcgaagtctccctcaacaccgagtaccaggecaacccggagac ggagtgatcaacctgaaggccgagccgacccgacccatcagcaacgcccggccctggagccggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcacccctggagttcgagctggtgtacgccgtaacaccaaccagac gatctccaagtcggttcgggaacctctcccttgggtcaaggcctggaggaccccgaggagtacctccgcatgggttcgaggtgtcc gcgtcctcttcttctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaaacaccgcatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtctactacaaggtgtacggcttgcggaccagaacatcctggagctgtacttaacgacg gcgacgtgtgttccaccaacaccttcatgaccaccgggaacgccctgggctccgtgaacatgacgacgggggtggacaacctgtt tatacgcacaagttccaggtgcgcgaggtcaagtgtTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCGCGCGGTTTGTGCCCCCTCGCGGGCGCCGCGGCCGCGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA

SEQ ID NO:	Nucleotide Sequence
	GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCGGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcggatagatcgcacactctggacgtggtcgtgatggac tgttgccgccacacttgctgacctgtgaatatccctgccgtttatcaaacagcctcagtgtgttgatcttggtgtacgcgttttg agttgctagctgcttgctattggaataccacccccagcatcccttccctgtttcatactgcttgcatccaaccgcaacttatctacgc tgtctgtatccctcagcgtgctcctgctcctgctcactgcccctgcacagccttggttgggtccgctgtattctcctggtactgcaacc tgtaaacagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatcaTTCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCAATTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAACCCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA TCGACCCCCGTGGGCCGGGCCCCCGGACCCCTATACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACCC AATGAGCGCGCTGAAGAAGGCCCTGTCCCTGCCAGCTTCCTGTTTTCTGTCTTCGTCT TCTACTGGAGCCTGCCAATCTTCGCCTCGGTGTACCGCATCCGCTTCGCGCGCGCTGGA AAGCGCAACGACATGCTGGACTGGGCTACCTCCCTGGTGGCGTTCTTTCGCGTGACCGT GCTCAAGCAGGGTCAGTTTTCGATCTACCGCGGAGGCCCCCTGCATCTACCTGTGCAACC ACCGTAGCTGGGCCGATTTCTTTATCGACATGTACCTACCGGAGGGCCGCGCCGCGTTC ATGTCCCGTTGGATGGTCTTCTTTGTCTTCCCCGTCTTCTGCTCCGCCGTGCTGATTCTG AAGGGCATTATCCTGTTTAAAGCGGGGCCGCATCCTGGACAAGGAGGCGTTTAAACGCCT GGCTGGACGCCACCCTGGCGAAGTCGACGATCCCCGGCCTGGTCGTGTACCCGGAGGG CCATCGCTCTCTGAAGCCGACGTGCTGCCCCCTGAAGCGCGGGATGCTGCACTACGCG TTTTCCCGTAAGCTCCAGTGCAACTGATCGTCACCCGCGGCAAGGAGGAGGTGCTGTC GGAGAAGACCCTGTCCATGCGCTTCGGGCGGACCCCTGGTGACCTCTTACTCGAAGGTG ATGAAGCCCAGCGACTACGGCTCCTTCGATGCCCTTATGACCGACGTGCACTGTGGCTG GGACCAAGTGTGGAAGGAGGCTACTCCGCGCCGACCCACGGCCTGCCGACGCTCTCG ATGGCTGGCGCCACCGAGTACGACTACCCCGCTTCCATGCGCTGGCAGCACCTGGCGA TGACGCTGTTTTCGATCGTCTGTTTGCGGGTGTCCTGGCCGCGAGCTGGTGGGGCGT CGGCGCCCTCCTGTCTGCTGCGGGCCCTACGGGCAAGCAGGTGGTCTGCTCCTGGCC TCGTGCTGGTTCGGCGCCAGCCTCCTGCGTAGCTTCTCTGTGAgtaccacgcccgcgcgcgcacctg acctgttctctcagggcgccctgtctgccttgcgaacaagccccgagcatgcgtcatgatcgtctcgcgccccgcgcgcggtttgt cgccctcgcggcgccccgcggcgccgcatggaattgtgcaaacccacctgacagattgagggccagcaggaagcggttg agatggaggtacaggagtcagtaactgaagttttatgataactaacaacaagggtcgttttgccagcgaatgacaagaacaagatcc acatttcgtgtagaggttgccatcgaatgtgagcggcgccgcccgcggaccggacaaacccctacgacgtggtagaaaaacgtggcgg

SEQ ID NO:	Nucleotide Sequence
	<u>gcactgtccctgtagcctgaagaccagcaggagacgatcggaagcatcacagcacaggccggcccgctctcgaacagagcgcgcg</u> <u>ccgcacgcatacctgcaggacagcgccatgccacgccccttfgatggcttcaagtacgattacgggtgttgattgtgtgtttgtgcgtagt</u> <u>gtgcatgggttagaataatacacttgattcttctcagggcaatctcggtgtcgaggttcaacccatttcggagtcaggtcag</u> <u>ccgcgcaatgaccagccgctacttcaaggactgcacgacaacgcccagggtgagctatgtttaggacttgattggaattgtcgtcga</u> <u>cgcattatcgcgctcgcgacagcaccgaagcaaaatgtcaagtgcgttccgatttcgctccgcaggtcgatgtgtgatcgtcgcg</u> <u>ccggatccgcccgtgtctcgtcgcttacgagctgaccaagcacccctgacgtccgggtacgcgagctgagattcgattagacataaat</u> <u>tgaagattaaaccgtagaaaaatttgatggtcgcgaaactgtgctcgattgcaagaaattgatcgtctccactccgaggtcgcca</u> <u>tcatcgagcaggcggtgtctccggcgcgccgctgggtgggggacagctgttctcgccatgtgtgtacgtagaaggatgaattt</u> <u>cagctgggtttcgttcacagctgtttgtgcatgattgtttcagactattgtgaatgttttagatttcttaggatgcatgattgtctgcat</u> <u>gcgact</u>

[0322] The construct can be written as

5'Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*VbolLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *VbolLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *VbolLPAAT2*. The initiator ATG and terminator TGA codons of the *VbolLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0323] **Table 83** shows the fatty acid/TAG profiles of eight primary transformants of CHK22 transformed with pCHK1214. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 83: Screen of primary transformants of CHK22 with pCHK1214.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1214-1	0.04	1.37	26.66	2.88	60.78	6.30	0.40	0.20	0.04	1.6
pCHK1214-2	0.04	1.47	29.88	2.83	56.72	6.87	0.53	0.21	0.00	2
pCHK1214-3	0.03	1.28	27.68	2.94	59.10	6.91	0.49	0.22	0.00	2
pCHK1214-4	0.03	1.17	25.97	2.91	61.34	6.48	0.49	0.23	0.00	1.6
pCHK1214-5	0.03	1.26	27.50	2.78	59.51	6.86	0.46	0.19	0.03	2.1
pCHK1214-6	0.03	1.31	27.66	2.85	58.78	7.24	0.52	0.21	0.03	2
pCHK1214-7	0.03	1.28	27.57	2.90	59.77	6.39	0.48	0.21	0.00	1.7
pCHK1214-8	0.03	1.31	27.93	2.98	58.75	6.92	0.50	0.22	0.03	2.1
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 40: Expressing *Pandorina morum* (*PmorLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0324] *PmorLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1215 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 84**.

Table 84: Integrative sequences for the transformation of *P. moriformis* with pCHK1215 encoding *PmorLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
70	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgccctccttggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcggttgccactctgcagagacggcaagtcgtccatcctctgcgtgtgtggcgcgacgtcgagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttcttaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgctccagatcgacagatagcgaccatgtgtgctgctgaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgtacaattctgtcaccatcgcatggagcaggtccacttagattcccgatc acccacgcacatctcgtaatagtcattcgtgtctctcgatcaatctcaagtgagtgcatggatcttggtgacgatcgcggtatg ggtttgcgccgtggctgcagggttgcccaaggcaagctaaccagctcctctccccgacaatactctcgaggcaaaagccggtca cttgccttcagattgccaataaactcaattatggcctctgcatgccatccatgggtctgatgaatggtcacgctcgtgctcgaccgtt ccccagcctctggcgtccctgccccgccaccagccccacgcgcggcagtcgctgccaaggtgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtgcaggccttctgttctgtgcccggcttcgcccaagatcagcg cctccatgacgaacgagacgtccgaccgccccctggtgcacttcacccccaaagggtgatgaacgacccccacggcctgtggtta

SEQ ID NO:	Nucleotide Sequence
	cgacgagaaggacgccaagtggcacctgtacttccagtagacaacccgaacgacaccgtctgggggacgcccttgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcccttctccggctccatgg tggtggactacaacaacacctecggcttcttaacgacaccatcgacccgcgccagcgtcgtggccatctggactacaacacccc gagtcgaggagcagtagatctcttacagcctggacggcggtacaccttaccgagtagcagaagaaccccgtgctggccgccaact ccacccagttccgcgacccgaaggtctctgtgtacgagccctccagaagtggatcatgaccgcgccaagtccaggactacaagat cgagatctactctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggttctcggctaccagtagagtgccc cggcctgatcagggtccccaccgagcaggacccagcaagtcctactgggtgatgttcatctccatcaaccccggcgccccggccggc ggctccttcaaccagtacttctcggcagcttcaacggcaccacacttcgaggccttcgacaaccagtcccgcgtgggacttcggcaag gactactacgacctgagaccttcttaacaccgacccgacctacgggagcgccctgggcatcgctgggacctcaactgggagtactc cgcttcgtgccccaccaacccctggcgctcctccatgtccctgtgcgcaagttctccctcaacaccgagtagcaggccaacccggagac ggagctgatcaacctgaagccgagccgacccgagccgacccgagccgagccgagccgagccgagccgagccgagccgagccgagccg gaagggccaacagctacaacgtcgacctgtccaacgacacccggcaccctggagttcgagctgggtgtacgccgtcaacaccaccagac gatctccaagtccgtgttcgagacctctccctgtgttcaaggccctggagaccccgagccgagccgagccgagccgagccgagccgagccg gcgtcctccttctcctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaacaccgcatgagcgtgaacaa ccagcccttcaagagcgagaacgacctgtctactacaaggtgtacggttctgtgaccagaacatcttgagctgtacttcaacgacg gcgacgtcgtgtccaccaacacactattcatgaccaccgggaacgcccctgggctccgtgaacatgacgacgggggtggacaacctgttc tactcgcacaagttccaggtgcgcgaggtcaagtgactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGATGATCG TCTCTGGCGCCCCGCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcgatagtagcagacactctggacgctggtgctgtgatggac tgttgcggccacacttgccttgacctgtgaatatccctgccgttttataacagccctagtggtttgatcttgtgtgtacgcgttttgcg agttgtagctgcttgccttatttgcgaatacaccceccagatcccccttccctgtttcatalcgttgcatcccaaccgcaacttatctacgc tgtctgtatccctcagcgtgctcctgtcctgtcactgcccctgcacagccttggttgggtccgctgtattctcctggtactgcaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtgggaggggaacacaaatggacntaggaataacaataTTCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTCTGGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCTGACGACTGTAACCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCGGGATCA TCGACCCCGTGGGCCGGGCCCCCGGACCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC

SEQ ID NO:	Nucleotide Sequence
	<u>A</u>ATGTCCCTGGTGACCAAGTTCCTGTCTCTGCCGTCGTTTCTCTTTTCGGTGTTCATCTT TTACTGGTCCCTGCCTATCTTCGCCATTCCATACCGCATCCGCTTCGCTAACGTGGGCA AGCGGAACGACATGCTCGACTGGGCGAAGGCTCTGGTGGGCTTCTTTTCGGTGTTCATCTT CCTGAAGCCCCGGCGCGACCTCCGTGTACCGCGGGGGTCCGTGCCTGTACCTCTGCAAC CATCGCTCCTGGGCCGACTTTTTCATCGACGTGTACCTGACCCAGGGCCAGTCGGCCCT CATGTGCGGGTGGCTGGTGTCTTTGTCTTTCCCGTGTCTGTACCTCCTGCCTGATCCT CCGCGGCATCGTCTCTTTAAGCGGGGCACGATCCTGGACAAGGAGGCGTTTAAACGCC TGGCTCGATGCTACGCGCGCCCCGAAGCATCGTGCCGGGGCTGGTGGTCTACCCGGAGG GGCATCGGTCCCTGAAGCCGACCTCGCTGCCGCTGAAGCGCGGGATGATCCACTACGC CTTCTCTCGGCGCTGCCAGTCCAACTGATCGTGACCTCGGGCAAGGAGCATGTGCTGA GCGAGAAGACGATGTGCGTCAAGTTTGGCAAGACCCTCGTGACCTCGTACTCGAAGCC GCTGAAGCCTAGCGATTACGCGACGTTTGATGCCTTCATGGCCGACATCCAGTCGAACT GGGACCAAGTGCTGGAAGGAGGCGTACACCGCTCCCGCTCGCGGCATGCCAGAGGTCTC CATGGCGGGCGCGCACTCTTACGCCTACCCACGCCTGACCTGGTGCCTCCAGTCGCC ATCACCGTCGTGTCCATCGTGATCCTGGGAGGGGTGTGGCGGGCTCGTGGCGGGGCG CCGCGGCCGTCTCGCTGCCGCGGGCCCCGTGGCCAAACAGGTGCTCGTCTCCCTGCT CGCCCTGTGGGCGGGGGTCTCCCTCCTGCGATCGTTCCTCTGA <u>gtaccacgccccgcgcgcacact</u> <u>gacctgttctctcgaggcgccctgttctgcttcggaacaaagccctggagcatgcgtcatgatcgtctgccccgccgcgcgtt</u> <u>gtcgccctcgcgccgccccgccgcggggcgccattgaaattgtgcaaacccacctgacagattgaggccccagcaggaaggcgtt</u> <u>gagatggaggtacaggagtaagtaactgaaagttttatgataactaacaacaaagggtcgttctgcccagcgaatgacaagaacaagattc</u> <u>cacatttccgtgtagaggcttccatcgaatgtgagcggcgggcgccgggacccgacaaaaccttacgacgtgtaagaaaaacgtggcg</u> <u>ggcactgtccctgtagcctgaagaccagcaggagacgacggaagcatcacagcagccggccccgcgtctcgaacagagcgcgccg</u> <u>gccgcatcgatacctgcaggacagcgccatgccacgcccctttagtgcttcaagtacgattacgggtgttgattgtgtgtgtgtgtag</u> <u>tgtgcatggtttagaataatacacttgatttctgtctacggcaatctcggttgcgcaggttcaacccatttcggagttcagggtca</u> <u>gccgagcaatgaccagcgctacttcaaggacttgcagcacaacgccagggtgagctatgtttaggacttgattggaaattgtcgtc</u> <u>acgcataattcgctccgacagcaccaagcaaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgtgtgatcgtcgcc</u> <u>gccggtacccggtgtctctgctgcttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattagacataa</u> <u>attgaagattaaacccgtagaaaaatttgatggtcggaactgtgctcgattgcaagaaattgatcgtctccactccgaggtcg</u> <u>catcatcgagcaggcggtgtctccggcgccggcgccgtggctgggggacagctgttctcgccatgtgtgtacgtagaaggatgaa</u> <u>tttcagctgggttttcgttcacagctgtttgtgcatgattgttcagactattgtgaatgttttagatttcttaggatgcatgattgtctg</u> <u>catgcgact</u>

[0325] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:PmorLPAAT2:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring

the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *PmorLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *PmorLPAAT2*. The initiator ATG and terminator TGA codons of the *PmorLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0326] Table 85 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1215. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 85: Screen of primary transformants of CHK22 with pCHK1215.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1215-1	0.04	1.28	27.52	2.88	59.31	6.54	0.46	0.25	0.00	1.96
pCHK1215-2	0.04	1.37	28.49	3.23	55.59	8.12	0.90	0.40	0.00	1.65
pCHK1215-3	0.04	1.34	27.96	2.94	58.55	6.86	0.49	0.27	0.00	1.56
pCHK1215-4	0.04	1.37	28.10	3.29	56.48	7.72	0.84	0.40	0.00	1.49
pCHK1215-5	0.04	1.43	27.16	2.28	60.66	6.01	0.28	0.17	0.00	1.68
pCHK1215-6	0.04	1.55	28.28	2.96	56.31	8.36	0.59	0.31	0.00	1.55
pCHK1215-7	0.04	1.35	28.28	2.87	57.63	7.31	0.52	0.27	0.00	1.63
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 41: Expressing *Volvox carteri* f. *weismannia* (*VcarfLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0327] *VcarfLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1216 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 86**.

Table 86: Integrative sequences for the transformation of *P. moriformis* with pCHK1216 encoding *VcarfLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
71	ccctcaactgcgacgtgggaaccttctccgggcaggcgatgtgcgtgggttgccctccttggcacggetctacaccgtcgagtacggc atgaggcggtgatggctgtgtcggttgcacttctgccagagacggcaagctgccatcctctgcgtgtgtggcgcgacgtcgagca gtccctctgcgacgatgagcgctgactttggccatttcacgcactcgagtgtacacaatccattttcttaagcaaatgactgctgatt gaccagatactgtaacgtgatttgcctccagatcgacagatagcgaccatgttgcgtcgctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattctctgtcacccatcggcgatggagcaggtccacttagattcccgatc accacgcacatctcgctaataatgcttctgtctgtcttcgatcaatctcaagtgcgtgtgcattgatcttgggtgacgatcggtgatg ggtttgcgcgctggctgcagggtctgcccaggcaagctaaccagctcctctcccgcacaatactctcgaggcaagccgggtca cttgccttcagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgctcgtgtctgaccgtt ccccagcctctggcgctccccgcccaccagcccacgcgcggcgagtcgctgccaaggctgtctcggaCTTCTCTGC GCTATGACACTTCCAGCAAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtcgaggccttctgttctgctggccggcttcgcccaagatcagcg cctccatgacgaacgagacgtccgaccgcccctgtgtcacttcacccccacaagggtggtgatgaacgacccaacggcctgtggt cgacgagaaggacgccaagtggcacctgtacttcagtaacaaccgaacgacaccgtctggggacgccctgttctggggccacgcc acgtccgacgacctgaccaactgggagaccagccatcgccatcgcccgaagcgcaacgactccggcgcttctccggctccatgg tggtggactacaacaacacctecggcttcttaacgacaccatcgaccgcgccagcgtgcgtggccatctggacctacaacccccg gagtccgaggagcagtacatctctacagcctggacggcggtacaccttcaccgagtaccagaagaaccccgctgtggccgccaact ccaccagttccgcgacccgaaggcttctgtgtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagtctgtgaagctggagtcgcgttcgccaacgagggcttctcgtggtaccagtacgagtggc cggcctgacgaggtccccaccgagcaggaccccagcaagtctactgggtgatgttcatctccatcaaccccgccgccccggccggc ggctccttcaaccgacttctgtcggcagcttcaacggcaccacttcgaggccttcgacaaccagtcccgctgggtggaactcgggcaag gactactacgccctgcagaccttcttaacaccgacccgaectacgggagcgccctgggcatcgctgggctccaactgggagtactc cgcttcgtgcccaccaaacccctggcgctcctccatgtccctcgtgcgaagtctcctcaacaccgagtaccagggcaaccgggagac ggagctgatcaacctgaaggccgagccgatcctgaacatcagcaacgcggccccctggagccgggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcacccctggaggttcgagctgggtgtacgccgtcaacaccaccagac gatctccaagtccgtgttcgcggacctctccctctggttcaaggccctggaggaccccgaggagtacctccgatgggttcgaggtgtcc gcgtcctccttctctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaccaaccgcatgagcgtgaacaa ccagccctcaagagcgaagacacctgtctactacaaggtgtacggcttctggaccagaacatcctggagctgtacttcaacgacg

SEQ ID NO:	Nucleotide Sequence
	gcgacgtcgtgtccaccaacacctacttcatgaccaccgggaacgccctgggctccgtgaacatgacgacgggggtggacaacctgttc tatatcgacaagttccagggtgcgcgagggtcaagtgactTAAGACGCCCCGCGGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCGCTTGCGAAACAAGCCCCTGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCCGCGCGGTTTGTGCGCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGGCCGCGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAAACCGGTgcagcagcagctcgatagatcgcacacactctggacgctggtcgtgtgatggac tgttgcgcgcacactgtctgttgacctgtgaatatccctgcgcgtttatcaaacagccctcagtgctgtttgactctgtgtacgcgcttttgcg agttgctagctgtgtgtctatttgcgaataccacccccagcatcccttcctcgtttcatatcgcttgcatcccaaccgcaacttatctacgc tgtctctctatccctcagcgctgtctctgtctctactgccccctgcacagccttggtttgggctcgcgctgtattctctgtgtactgcaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatcaTTTCGCCG CTCAGCCTACACGCTCTCTCCGATACCTTTCCCTCATTGCATTTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGCACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCCTGACGACTGTAACCCCTGCCAGAAACAATTG AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA TCGACCCCGTGGGCGGGGCCCCCGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC AATGAGCATTACCTCGAAGGCTCTGAGCCTGCCTTCCTTCCTGTTACACGTGTTCTGTCT TCTACTGGTCCCTGCCTCTCTTCGCCATTATCTACCGCATCCGCTTTGCCAACGTGGGC AAGCGGAACGATATGCTGGATTGGGCTAAGGCGCTCGTCGCCTTTTTCCGCGTCACCGT CCTGAAGCAGGGTGAGATGTCCCTGTACCGCGGAGGCCGCTGCCTGTACCTGTGCAAC CACCGCTCCTGGGCCGACTTTTTCATCGACACCTACCTGACGGAGGGACGCGCCGCGC TCATGTCTCGGTGGATGGTGTTTTTCGCCTTTCCCGTGTTTTCCACGTCCGTCTGATCC TGCGCGGCATCGTCTGTTTAAGCGGGGCCATATCGCCGACAAGGAGGCCTTTAACTCT TGGCTCGACGCCACCCTGCGGAAGTCGACGGTGCCAGCCCTGCTCGTCTACCCGGAGG GACACCGCTCTCTGAAGCCGCGCTCCCTGCCCCCTCAAGCGCGGTATGCTGCATTTCCGG TTCTCCCGCCAGCTCGCGGTGCAGATGGTCATCACGCGAGGCAAGGAGGAGGTGCTGA GCGAGAAGACGTGCTCGGCCCCGTGGGGCCGCACCCTGGTCACCACGTACTCCAAGGT GATCAAGCCCCACGGACTACGACTCCTTTGACGCTTTTTTCAACGAGATCCAACCACGT GGGACGCCCTGCTGGCAACTGTCGTACCACCAGCCCCACGAGGGCGTGCCCCAACTGTC GCTGAAGGGCGCGCACGAGTACGACTACCCGGTGAAACATGTGGTGCTGCAACTGGGC GTCACGCTCCTGTCCATCACGATCCTGGTGGCGGTCTGTACGGGAGCTGGGTGGCGC

SEQ ID NO:	Nucleotide Sequence
	<i>TGCGCGGGCGTCTCTTCCCTGGGCAGCTCCATCACGCAACAGGCGGTGGTCTCTGGG</i> <i>CGTGGCGGGCTGGCTCGGCGTGTCCCTCCTGCGGTGTTTCTGTGA</i><u><i>gtaccacgcccgcgcggc</i></u> <u><i>gcacctgacctgttctctgagggcgctgttctgcttgcgaacaagccccggagcatgctgcatgacgtctctggcggcccgccgcg</i></u> <u><i>cggtttgcgcccgcgcggcgccccggcgcgggggcgcatgaaattgttgcaaacccacctgacagattgaggccaggcaggaa</i></u> <u><i>ggcgttgagatggaggtacaggagtcagtaactgaaagttttatgataactaacaacaaagggtcgttctgcccagcgaatgacaagaac</i></u> <u><i>aagattccacatttccgttagaggcttgcctcgaatgtgagcgggcgggccgcggacccgacaaaacccttacgacgtggttaagaaaaa</i></u> <u><i>cgtggcgggcactgtccctgtagcctgaagaccagcaggagacgacggaagcatcacagcacagggcgcccgctctcgaacagagc</i></u> <u><i>gcggcgggcgcatcgatacctgcaggacagcgccatgccacgccccttggatggctcaagtacgattacgggttggtgtgtgtgtgt</i></u> <u><i>gcgtagtgtgcatggtttagaataatacaacttgatttcttctcacggcaatctcggttgcgcagggtcaacccccatttcggagtctc</i></u> <u><i>aggtcagccgcgaatgaccagccgctacttcaaggacttgcacgacaacccgagggtgagctatgttaggacttgattggaaatt</i></u> <u><i>gtcgtcgacgcataattcgctccgcgacagcacccaagcaaaatgtcaagtgcgttcgatttgcgtccgaggtcagtggtgtgac</i></u> <u><i>gtcgcgccggatccgcccgtgtctctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattaga</i></u> <u><i>cataaattgaagattaaacccgtagaaaaattgatggtcgcaactgtctcgattgcaagaaattgatgctctccactccgcag</i></u> <u><i>gtcgccatcatcgagcaggcggtgtctcccgccggcgccgtgggtggggggacagctgtctcgccatgtgtgtacgtagaagg</i></u> <u><i>atgaatttcagctggtttcgttgacagctgtttgtcatgattgtttcagactattgttgatgttttagattcttaggatgcatgatt</i></u> <u><i>gtctgcatgcgact</i></u>

[0328] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:*VcarfLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *VcarfLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *VcarfLPAAT2*. The initiator ATG and terminator TGA codons of the *VcarfLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0329] Table 87 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1216. The *P. moriformis* base strain CHK22 is shown as a non-transgenic

control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 87: Screen of primary transformants of CHK22 with pCHK1216.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1216-1	0.04	1.34	28.27	2.83	57.48	7.53	0.49	0.25	0.00	3.20
pCHK1216-2	0.04	1.36	28.16	2.76	57.88	7.41	0.47	0.24	0.00	3.25
pCHK1216-3	0.04	1.40	28.56	2.78	56.79	8.04	0.50	0.25	0.00	3.38
pCHK1216-4	0.04	1.37	28.36	2.82	57.35	7.67	0.50	0.25	0.00	3.11
pCHK1216-5	0.04	1.31	28.00	2.96	58.66	6.65	0.49	0.27	0.00	1.56
pCHK1216-6	0.04	1.31	26.65	2.34	58.33	8.80	0.50	0.22	0.00	3.36
pCHK1216-7	0.04	1.40	28.38	2.67	57.13	7.93	0.50	0.24	0.00	3.10
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 42: Expressing *Eudorina cylindrica* (*EcyLLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0330] *EcyLLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1217 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 88**.

Table 88: Integrative sequences for the transformation of *P. moriformis* with pCHK1217 encoding *EcyLLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
72	ccctcaactgcgacgtgggaaccttctccgggcaggcgatgtgcgtgggttgccctcttgccacggctctacaccgtcagtagcgc atgaggcggtgatggctgtgtcggttgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgctgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcagtgtagacaaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgtccagatcgacagatagcgaccatgtgtgctgctgtaaaatctggattccgaattcgacc ctggcgctccatccatgaacagatggcgacactgtttacaattctgtcaccatcgccatggagcaggtccacttagattcccgatc accacgcacatctcgctaatagtcatttcgtgtcttcgatcaatctcaagtgagtgatggatcttggttgacgatcggtatg ggtttgcgccgtggctgcagggttgcccaaggcaagctaacccagctcctctccccgacaatactctgcaggcaaaagccggtca cttgcccttcagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgctcgtgtcctgaccgtt ccccagccttggcgtccccgccccaccagcccaagccgcggcgagtcgctgccaaggtgtctcggaCTTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT

SEQ ID NO:	Nucleotide Sequence
	<u>CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC</u> <u>ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA</u> <u>GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA</u> <u>GTCACAACCCGCAAACCTGTACA</u>ATGctgtgcagggccttctgttctgtgcccgttcgccccaagatcagcg cctccatgacgaacgagacgtccgacgcgccctggtgcacttcaccccaacaaggctggatgaacgaccccaacggcctgtggtg cgacgagaaggacgccaagtggcacctgtacttccagtacaacccgaacgacaccgtctggggacgccctgttctggggccacgc acgtccgacgacctgaccaactgggaggaccagcccatcgccatgccccgaagcgcaacgactccggcgcccttccggctccatgg tggtggactacaacaacacctcggcttctcaacgacaccatcgacccgcgcagcgctgctggccatctggactacaacaccccg gagtcggaggagcagtagatctctacagcctggacggcggttacaccttaccgagtagcagaagaaccccggtgctggccccaact ccaccagttccgcgacccgaaggcttctgtgtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagctctggaagctggagtcgcgttcgccaacgagggcttctcggctaccagtagcagtgccc cggcctgatcgaggtccccaccgagcaggacccagcaagtctactggtgatgttcatctccatcaaccccgcgccccggccggc ggctccttcaaccagtagtctgtgcgagcttcaacggcacccacttcgagccttcgacaaccagtcccgcgtggtgacttcggcaag gactactagccctgcagaccttcttcaacacgacccgacctacgggagcgccctgggcatcgctgggctccaactgggagtactc cgcttcgtgcccaccaacccctggcgctctccatgtccctgtgcgaagtctccctcaacaccgagtaccaggccaacccggagac ggagctgatcaacctgaagccgagccgacgtctgaacatcagcaacgcccggccctggagccggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcaccctggagttcgagctggtgtacgccgaacaccaaccagac gatctcaagtcggttcgagacctctccctgtgttcaaggccctggaggaccccgaggagtacctccgcatgggttcgaggtgtcc ggctctctcttctctgaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttaccacacccgatgagctgaacaa ccagccctcaagagcgagaacgacctgtctactacaaggtgtacggctgtgtaggaccagaacatcttgagctgtacttcaacgacg gcgagctgtgtccaccaacacacttctcatgaccaaccgggaacgcccgtgggtccgtgaacatgacgacgggggtgacaacctgttc tatatcgacaagttccaggtgcgcgaggtcaagtgtactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcgatagatcgaacactctggacgctggtcgtgtgatggac tgttgcggccacactgtgctccttgacctgtgaatctcctgcgctttatcaaacagcctcagtggtttgatcttgtgtgtacgcgcttttgcg agttgctagctgcttgtctatttgcgaatacaccceccagcctcccttccctgttcatatcgttgcatcccaaccgcaacttatctacgc tgtctgtatccctcagcgctgctcctgtcctgtcactgcccctgcacagccttggttgggctccgctgtattctcctggtactgaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtggtgggaacacaaatggacntaggaataacaatcaTTCGCGG CTCAGCCTACAGTCTTCTCCGATACCTTCCCTCATTGCATTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCTGACGACTGTAACCTGCCAGAAACAATTC

SEQ ID NO:	Nucleotide Sequence
	AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGCG TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGCGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA TCGACCCCGTGGGCCGGGCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC AATGTCCCTGATCTCGAAGGCTCTGTGCTCCCGTCTTTCTCTTTTCGGTCTTCGTGTT CTACTGGTCGCTGCCTTTTTTCGCCACCACGTACCGCATCCGCTTCGCCAACGTGCGTA AGCGAAACGACATGCTGGACTGGGCTAAGACGCTGGTCGCGTTTTTCGCGTCAACCGT CCTGAAGCAGGGCAACATGTCCCTCTACCGTGGGGGCCGCTGCCTGTACCTGTGCAAC CACCGCTCCTGGGCCGACTTTTTTCATCGACACCTACCTGACGGAGGGCCGCGCGGCC TGATGTCCCGGTGGCTCGTGTACTTTGTCTTCCCGTGTCTCTGCACGAGCGTGATTATC CTCCGAGGGATCGTGCTGTTTAAGCGTGGCCACATCGCGGACAAGGAGGCTTTAACG CGTGGCTCGACGCCACGTTCTCCCGCAGCATCGTGCCGGGCCTCCTGGTGTACCCCGA GGGGCACCGCTCCCTGAAGCGCACCTCTCTGCCCCTGAAGCGGGGTATGATGCACTAC GCTTACTCGCGCCGAATCCCCGTGCAACTGATCGTGACCCGCGGCAAGGAGGAGGTCC TGTCGGAGAAAGACCTCTCGGCGCGGTGGGGCCGAACCTGGTCAACGACCTACAGCAA GGTCTGAAGCCGTCCGACTACGACTCCTTCGACGCCTTCTTTAACGACATCCAGGCGC AGTGGGACGCCCTGCTGGAAAGACGAGCTACGACACCCACGCCGCGGGCGTCCCGCAGCT CTCCATGGTCGATGCCCACGAGTACGACTACCCCGTCGAATGTGGTGGCTGCAACTG GGCATGACCTCTCTGTCCATCGTGATCCTGGCCGCGGTCTGTGGGGCTCCTGGCTGG GCATCCGTGCGGGCTGGCCACGCTGGGCTCCCTCCAGCAACAGGCCGTCGTGTGGGG CGTTCCGGCTGGCTGGGCGCCTCGCTCCTGCTCTCGTTCTTCTGA <u>gtaccagccccgcgcgcgc</u> <u>cacctgacctgtctctc</u><u>gaggcgccctgtctgccttcgaaacaagccctggagcatgcgtcatgatcgtctctgcgcgcccgccgcgc</u> <u>ggtttgtcgccctcgcggcgcccccgccgcggggcgccattgaaattgttgcaaacccacctgacagattgaggccccagcgaggaa</u> <u>gcgttgagatggaggtacagagatcaagtaactgaaagttttatgataactaacaacaagggtcgtttctgccagcgaaatgacaagaaca</u> <u>agattccacatttccgtgtagaggcttgcacatgaatgtgagcggcgccgcgcgaccgcacaaaaccccttacgcgtgtgaagaaaaac</u> <u>gtggcgggcactgtccctgtacctgaagaccagcaggagacgatcggaagcatcacagcacaggccgccccgcgtctcgaacagagcg</u> <u>cggcgcccgcatcgatacctgcaggacagcgccatgccacgccccttgatggcttaagtacgattacgggtgttgattgtgtgtttgttg</u> <u>cgtagtgtgatgtgttagaataatacacttgatttctgtctacggcaatctcggttgcgcaggttcaaccccaatttcggagtctca</u> <u>ggtcagccgcgaatgaccagccgctacttcaaggacttgcagacaacgccgaggtgagctatgtttaggacttgattggaattgt</u> <u>cgtcagcgcatttcggtccgcgacagcacccaagcaaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgttgtatcgt</u> <u>cggcgccggatccgcggctgtctctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattagac</u> <u>ataaattgaagattaaaccctagaaaaatttgatggtcgcaaaactgtctcgattgcaagaaattgatcgctccctcactccgcagg</u> <u>tcgcatcatcgagcaggcggtgtctccggcgggcgccgtgctgtgggggacagctgtctcggccatgtgtgtacgtagaagga</u> <u>tgaatttcagctggttttcgttcacagctgtttgtgcatgattgtttcagactattgtgaatgttttagattcttaggatgcatgatttg</u> <u>tctgcatgcgact</u>

[0331] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:*EcyLLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *EcyLLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *EcyLLPAAT2*. The initiator ATG and terminator TGA codons of the *EcyLLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0332] **Table 89** shows the fatty acid/TAG profiles of eight primary transformants of CHK22 transformed with pCHK1217. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 89: Screen of primary transformants of CHK22 with pCHK1217.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1217-1	0.03	1.25	27.10	2.86	60.21	6.45	0.48	0.22	0.00	1.7
pCHK1217-2	0.04	1.29	26.09	2.51	59.66	8.12	0.51	0.20	0.04	1.8
pCHK1217-3	0.03	1.31	27.08	3.21	60.00	6.33	0.50	0.22	0.00	1.6
pCHK1217-4	0.03	1.29	27.33	3.04	59.62	6.59	0.50	0.23	0.03	1.5
pCHK1217-5	0.04	1.47	27.55	2.50	58.97	7.06	0.57	0.21	0.05	1.6
pCHK1217-6	0.03	1.19	26.65	2.91	60.50	6.60	0.49	0.24	0.03	1.7
pCHK1217-7	0.03	1.24	26.95	3.00	60.13	6.55	0.50	0.24	0.03	1.6
pCHK1217-8	0.04	1.33	27.76	2.84	57.86	7.86	0.60	0.25	0.00	2
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 43: Expressing *Gonium multicoccum* (*GmulLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0333] *GmulLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1218 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 90**.

Table 90: Integrative sequences for the transformation of *P. moriformis* with pCHK1218 encoding *GmulLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
73	ccctcaactgcgacgctgggaaccttctccgggcaggcgatgtgcgtgggttgcctcctggcagcgctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgccatcctctgcgtgtgtggcgacgctgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgctccagatgcacagatagcgaccatgttgcgtgcgtgaaaatctggattccgaattcgacc ctggcgctccatccatgaacagatggcgacacttgttacaattcctgtcacccatcgccatggagcaggctccacttagattcccgatc acccacgcacatctcgtaatagtcattcgttctgtcttcgatcaatctcaagtgcgtgtgcattggttgacgatgcggtatg ggtttgcgcgctggctgcagggtctgccaaggcaagctaaccagctcctctcccgcacaatactctcgaggcaaaagccggtca cttgccttccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgctcgtgctcgaccgtt ccccagccttggcgctcccctgccccgccaccagccacgcgcggcgagtcgctgccaaggctgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtgcagccctcctgttctgtggtgcggcttcgccaagatcagcg cctccatgacgaacgagacgtccgacgcgccctggtgcacttaccaccaacaaggctggatgaacgacccaacggcctgtgta cgacgagaaggacccaagtggcactgtacttcagtaacccgaacgacaccgtctggggacgccctgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatgcgccgaagcgcaacgactccggcgccttctccggtccatgg tggtggactacaacaacacctcggcttcttaacgacacccatgcacccgcgcagcgctgcgtggccatctggacctacaacaccccg gagtcgaggagcagtagatctctacagcctggacggcggtacaccttcaccgagtaccagaagaaccccgctgtggccccaact ccaccagttccgcgacccgaaggcttctgtgtacgagccctccagaagtggatcatgaccgggccaagtccaggactacaagat cgagatctactcctccgacgacctgaagctctggaagctggagtcgcgttcgccaacgagggttctcggctaccagtagagtgccc cggcctgatcgaggtccccaccgagcaggacccagcaagtctactgggtgatgttcatctccatcaaccccgcgccccggcgcc ggctccttaaccagtaacttcgtcggcagcttcaacggcacccattcgaggccttcgacaaccagtcggcggtgtggacttcggcaag gactactacgccctgcagacttcttaacacccgacccacgagcgagcgccctgggcatcgctgggctccaactgggagtactc cgcttctgtgccaccaacccctggcgctcctcatgtccctgtgcgcaagtctccctcaacaccgagtaccaggecaacccggagac ggagctgatcaacctgaaggccgagccgatcctgaacatcagcaacgcggccctggagccggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtcaacagcaccggcaccctggagttcgagctggtgtacgccgaacaccaaccagac gatctcaagtcggttcgggacctctccctctgtttcaaggcgctggaggaccccgaggagtacctccgatgggttcgaggtgtcc

SEQ ID NO:	Nucleotide Sequence
	g c g t c c t c c t t t c t c t g g a c c g c g g g a a c a g c a a g g t g a a g t c g t g a a g g a g a a c c c t a c t t c a c c a a c c g c a t g a g c g t g a a c a a c c a g c c c t t a a g a g c g a g a a c g a c c t g t c t a c t a c a a g g t g t a c g g t t g c t g g a c c a g a a c a t c c t g g a g c t g t a c t t c a a c g a c g g c g a c g t c g t g t c c a c c a a c a c c t a c t t c a t g a c c a c c g g g a a c g c c c t g g g t c c g t g a a c a t g a c g a c g g g g t g g a c a a c c t g t t c t a c a t c g a c a a g t t c a g g t g c g c g a g g t c a a g t g a c t T A A G A C G C C C G C G C G G C G C A C C T G A C C T G T T C T C T C G A G G G C G C C T G T T C T G C C T T G C G A A A C A A G C C C C T G G A G C A T G C G T G C A T G A T C G T C T C T G G C G C C C C G C C G C G C G G T T T G T C G C C C T C G C G G G C G C C G C G G C C G C G G G G C G C A T T G A A A T T G T T G C A A A C C C C A C C T G A C A G A T T G A G G G C C C A G G C A G G A A G G C G T T G A G A T G G A G G T A C A G G A G T C A A G T A A C T G A A A G T T T T A T G A T A A C T A A C A A C A A A G G G T C G T T T C T G G C C A G C G A A T G A C A A G A A C A A G A T T C C A C A T T T C C G T G T A G A G G C T T G C C A T C G A A T G T G A G C G G G C G G G C C G C G G A C C C G A C A A A A C C C T T A C G A C G T G G T A A G A A A A C G T G G C G G G C A C T G T C C C T G T A G C C T G A A G A C C A G C A G G A G A C G A T C G G A A G C A T C A C A G C A C A A C C G G T g c a g c a g c a g c t c g g a t a g t a t c g a c a c a c t t g g a c g t g t g t g a t g g a c t g t t c c g c c a c a c t t g t g c t t g a c c t g t g a a t a t c c c t g c c g t t t a t c a a a c a g c c t c a g t g t g t t g a c t t g t g t a c g c g t t t g c g a g t t g t a g t g c t t g t g c t a t t t g c a a t a c c a c c c c a g a t c c c t t c c t g t t c a t a t c g t t g c a t c c a a c c g c a a c t a t a c g c t g t c t g t a t c c c t c a g e g t g e t c c t g t c t c t g t c a c t g c c c t c g c a c a g e c t t g g t t g g g c t c c g c t g t a t t c t c t g t a c t g c a a c c t g t a a c c a g c a c t g c a a t g t g a t g c a c g g g a a g t a g t g g g a t g g g a a c a c a a t g g a c c t a g g a a t a a c a a t c a T T C G C C G C T C A G C C T A C A C G T C T T C T C C G A T A C C T T T C C C T C A T T G C A T T T A T G C C A G A C T G G G T C C C A G C C T G G G T G G G T G C T C C C G C T C G A T T G C T C G T G T C G G A G G C G G G G C A C C C C G C T C T C T C T A T T T A T C A C T G C C T C T C C C C G A C C A A C C C T G A C G A C T G T A A C C C T G C C A G A A A C A A T T C A G C C T C A A C A A G C C G A G T T G T G C A C A A G G G A G A C T A A T T T T T A G A C G G G A A G C A A C C C G C T T C C A G A A G C A T C C G G A C G G G G T A G C G A G G C T G T G T C G A G C G C C G T G G G G A T C T G G C C G A T G A G G T G C C C G A A A C C C G T G T A C A G C T C A G C G G C T G G G A T C A T C G A C C C C G G G A T C A T C G A C C C C G T G G G C C G G G C C C C G G A C C C T A T A A C T A A A A G C C G A C G C C A G C G A A A A C C A C A A A C A T T T A C T C C T T A A T C C T C G C T C C T C C T T C A T A T A C A A C C A C A A G T A A T C A A C T C A C C A A T G G G T G C G G C C G A A A A G C T G C T C T C C C T C C C A T C C T T T C T G T T C A G C G T G T T T G T C T T T T A C T G G A G C C T G C C A A T C T T C G C G T G G T G T A C C G T A T C C G A T T C G C C A G C T C C G G C C G A C G C A A C G A C A T G C T C G A C T G G G C T A A G A G C A T C G T C C G G T T C T T T G G C G T G A C C G T C G T G A A G G T G G G C G A G A A C A G C C T G T A C C G C G A G G G G C C A T G T C T G T A C C T G T G C A A C C A C C G C T C G T G G G C C G A T T T T T C A T C G A C G C T T A C C T C A C C G A G G G A C G A G C G G C C C T G A T G T C G C G T G G C T G G T G T T T T C G T G T T T C C G T G T T C T G C A C C T C C T G C C T G A T C C T G C G C G G C A T C G T C C T C T T C A A G C G G G G C A C C A T C G C C G A C A A G G A G G G C T T C A A C C G C T G G C T C G A C T C C A A G C T G G G C T C C T C G C A C G T G C C T G G T C T C C T G G T C T A C C C T G A G G G C C A C C G C A G C C T C C G C C C C T C T T C G C T G C C G C T G A A G C G C G G T A T G T C C A C T A C G C C T A C G G C C G A A G C T C G C C G T G C A G A T T A T C T G C A C G G C G G G G A A G G A G G G G T G A T G T C G G A G A A G A C C A T G G C C G T G C A C T T C G G C C G A C G C T C G T C A C G A C C T T C A G C C A G G T C C T G C G C C A T C T G A G T A C C C C A C C T T C G A C G C C T T T T C G C G G C C G T G C A G T C C G C G T G G G A T G C G A G C T G G A A G G C C G C G A A C C A G A C C C C C T C G G C G G G C C T G C C C C G C C T G

SEQ ID NO:	Nucleotide Sequence
	<i>TCCATGGAGGGAGCGCAGGACTACGACTACCCGCCCGCGATGCGCGCGCAGCAACTGG</i> <i>GAGTGACCCCTCTGAGCTTCGTGGTCTTCGGAGGCGCCCTGCTCGTCACGTGGCGACT</i> <i>CCTGGCTGCCCTCTGGCGGCTGCGGGTGGGCCCCAACAGGCTGTGCCCTGCTCTG</i> <i>GCGGGCTGGACGGGCGTCTCTATGCTCCGCAGCTTCCTGTGA</i><u>gtaccacgccgcgcgcacctg</u> <u>acctgttctcgcaggcgccctgttctgccttcgaaacaagccccctggagcatgcgtgcgatgcgtctctggccccgccgcggtttgt</u> <u>cgcctcgcggcgccccggcgggcgccattgaaattgttgcacacccacctgacagattgaggccccagcaggaaggcggtt</u> <u>agatggaggtacaggagtaagtaactgaaagtgtttatgataactaacaacaagggtcgttctggccagcgaatgacaagaacaagattcc</u> <u>acatttcggtgtagaggttgcctcgaatgtgagcggcgccgcggaccgcacaaaacccttacgacgtgtaagaaaacgtgcgg</u> <u>gcactgtccctgtagcctgaagaccagcaggagacgatcgaagcatcacagcagccgcgccgcgtctcgaacagagcgcgcgg</u> <u>ccgcctgcatactgcaggacagcgccatgccacgccttggatggcttcaagtacgattacgggtgttgattgtgtgtttgttcgtagt</u> <i>gtgcatggtttagaataatacattgatttctgctacggcaatctcggttgcgcaggttcaacccatttcggagtctcaggtcag</i> <i>ccgcgaatgaccagccgctacttaaggacttgcacgacaacgccgaggtgagctatgtttaggacttgattggaaattgtcgtcga</i> <i>cgcatattcgctccgcgacagcaccgaagcaaaatgtcaagtgcgttccgattgcgtccgcaggtcagtggtgtgctcgcg</i> <i>ccggatccgcgggtgtgtcctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattagacataaat</i> <i>tgaagattaaacccgtagaaaaattgatgtgcgaaactgtgtcgtgattgcaagaaattgatcgtctccactccgaggtcgca</i> <i>tcacgagcaggcggtgtcctccggcgggcgccgtggtggggggacagctgttctggccatgtgtgtacgtagaaggatgaattt</i> <i>cagctggtttcgttgacagctgtttgtgcatgattgtttcagactattgtgaatgttttagatttcttaggatgcatgattgtctgcat</i> <i>gcgact</i>

[0334] The construct can be written as 5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:GmulLPAAT2:PmPGH::3'Thia4. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the GmulLPAAT2 gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the GmulLPAAT2. The initiator ATG and terminator TGA codons of the GmulLPAAT2 are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0335] **Table 91** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1218. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 96 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 91: Screen of primary transformants of CHK22 with pCHK1218.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1218-1	0.04	1.51	28.72	3.37	52.27	11.15	1.14	0.29	0.00	3.9
pCHK1218-2	0.03	1.29	26.95	3.30	59.47	6.85	0.50	0.27	0.03	1.6
pCHK1218-3	0.03	1.32	27.80	2.83	57.95	7.92	0.51	0.22	0.00	3.7
pCHK1218-4	0.03	1.33	27.71	2.75	57.81	8.21	0.51	0.21	0.00	4.2
pCHK1218-5	0.03	1.33	27.58	3.13	57.84	7.97	0.52	0.24	0.00	3.8
pCHK1218-6	0.04	1.59	28.47	2.82	56.81	8.40	0.33	0.19	0.00	3.5
pCHK1218-7	0.04	1.37	28.24	2.83	57.34	8.03	0.52	0.21	0.00	3.9
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 44: Expressing *Gonium viridistellatum* (*GvirLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0336] *GvirLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1219 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 92**.

Table 92: Integrative sequences for the transformation of *P. moriformis* with pCHK1219 encoding *GvirLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
74	ccctcaactgcgacgctgggaaccttctccgggcaggcgatgtgcgtgggttgccctctggcagcgctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgctgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgtccagatgcacagatagcgaccatgttgcgtcgtctgaaaatctggattccgaattcgacc ctggcgctccatcatgcaacagatggcgacacttgttacaattcctgtcacccatcgcatggagcagggtccacttagattcccgatc acccagcacatctcgtaatagtcattcgttctgtcttcgatcaatcgaagtgtgcatggtatcttggttgacgatgcggtatg gggttgcgcgctggctgcagggtctgccaaggcaagctaaccagctcctctcccgcacaatactctcgaggcaaaagccggtca ctgacctccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgctcgtgctgacggtt cccagcctctggcgtccctgccccgccaccagccacgcgcggcagtcgctgccaaggtctctcggaCTTCTTGC

SEQ ID NO:	Nucleotide Sequence
	<u>GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC</u> <u>ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT</u> <u>CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCGATTGCAAAGAC</u> <u>ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA</u> <u>GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA</u> <u>GTCACAACCCGCAAACCTGTACA</u>ATGctgtcagggccttctgttctgtgtggcggcttcgccccaagatcagcg cctccatgacgaacgagacgtccgacggccccctgggtgacattcaccccaacaaggctggatgaacgaccccaacggcctgtggta cgacgagaaggacgccaagtggcacctgtacttccagtacaacccgaacgacacggctgggggacgccctgtgtgggcccacgcc acgtccgacgacgtgaccaactgggaggacagcccatcgccatcgccccgaagcgcaacgactccggcgcccttccggctccatgg tggtggactacaacaacacccctcggttcttaacgacacccatcgacccgcggcagcgctgcgtggccatctggactacaacaccccg gagtcaggagagcagtagatctcctacagcctggacggcggtacaccttcaccgagtagcagaagaaccccggtgtggcgccaact ccaccagttccgcgacccgaaggtctctgtgtagcagccctccagaagtggatcatgaccgcggccaagtcccgagactacaagat cgagatctactcctcgacacgtgaagtcctggaagctggagtcgcgttcgccaacgagggttctcggctaccagtacgagtgccc cggcctgacgaggtccccaccgagcaggaacccagcaagtcctactgggtgatgttcatctccatcaaccccggcgccccggcgcc ggctccttcaaccagtagtctcgtcgagcttaacggcacccacttcgaggtcctcgacaaccagtcgccgtgtggacttcggcaag gactactacgccctgcagaccttcttaacacccgacccgacctacgggagcgccctgggcatcgctgggctccaactgggagtactc cgcttctgtgcccaccaacccctggcgctcctccatgtcctcgtgcgcaagttctccctcaacaccgagtagcaggccaacccggagac ggagctgatcaacctgaaggccgagccgacgctgaacatcagcaacgcccggccctggagccgttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacgacacccggcacccctggaggttcgagctgggtgtagccgtaacaccaccagac gatctccaagtccgtgttcgagacctctccctctgtgttcaaggccctggagaccccgaggagtagctccgcatgggcttcgaggtgtcc gcgtcctcttcttctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaccaaccgcatgagcgtgaacaa ccagcccttcaagagcgagaacgacctgtcctactacaaggtgtacgcttctgtgaccagaacatcctggagctgtacttcaacgacg gcgacgtcgtgtccaccaacacctaattcatgaccaccgggaacgcccctgggtccctgtgaacatgacgacgggggtggacaacctgttc taccatcgacaagttccaggtgcgcgaggtcaagtactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGATGATCG TCTCTGGCGCCCCGCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAAACCGGTgcagcagcagctcggatagatcgacacactctggacgctgtgtgtgatggac tgttgcggccacacttctgcttgaacctgtgaatatccctgcgctttatcaaacagcctcagtgtgttgcattgtgtgtacgcgcttttgcg agttgtagctgtgtgtctatttgcgaatacaccacccagcatcccttccctcgtttcatatcgcttgcacccaaccgcaacttatctacgc tgtctgtatccctcagcgtctcctcgtcctgtcactgcccctgcacagccttggttgggtccgctgtattctcgtgtactgcaacc tgtaaacagcactgcaatgtgtgacacgggaagtagtggtggtggaacacaaatggactaggaaataacaatcaTTCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTATGCCAGACTGGGTCCC

SEQ ID NO:	Nucleotide Sequence
	<u>AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGGAGGCGGGGACCCCCGCTCTC</u> <u>TCTATTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAACCCTGCCAGAAACAATT</u> <u>AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC</u> <u>TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGCGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCCAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TCGACCCCGTGGGCCGGGCCCCCGGACCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTACTCCTTAATCCTCGCTCCTCCTCATATACAACCACAAGTAATCAACTCACC</u> <u>A</u>ATGGGTGCGGCCTCGAAGCTCCTGGGCCTGCCATCCTTCCTGTTCTCCGTCTTCGTCT TTTACTGGTCCCTGCCATCTTCGCCATCGTCTACCGCATCCGCTTCGCTTCCCCAGGG CGGCGCAACGACATGCTGGAAGTGGGCAAGTCGATTGTGGGTTTTTCGGCGTGCGCCG TCCACAAGGCGGGTACGAACAGCCTGTACCGAGACGGCCCGTGCCTGTACCTCTGTAA CCACCGCTCTTGGGCGGACTTTTTCATCGACGCCTACCTACGGAGGGCCGTGCGGCTC TGATGTCGCGCTGGCTGGTCTTTTTCGTGTTCCCCGTGTTCTGCACGAGCTGCCTGATC CTCCGCGGTATCGTCTCTTCAAGCGCGGCACCATCGCGGACAAGGAGGCCTTAAATAG CTGGCTGGACGCTACGCTCGGCCGCTCCATCGTGCCCGGTCTCCTGGTCTACCCCGAG GGCCACCGTTCCCTGCGCCCCGCTCCCTGCCGCTGAAGCGCGGCATGCTGCACTACG CTTTTGGTCGCAAGCTGCCCCTGCAGGTGGTCTGCACCGCTGGCAAGGAGGGAGTCAT GTCGGAGAAGACCATGGCTGTGCGCTTCGGCCGTCGCTCGTACCAACTTCTCCAG GTGATCCGCCCTGCCGACTTCCCAACGTTTCGACGCCTTCAGCGCGGCCGTCCAGTCCGC CTGGGACGGCTGCTGGAAGGCGGCCTACGAGACCCCCGCTGCGGGCCTCCCTCGCCTG TCCATGGAGGGCTCGCATGAGTACGAGTACGACTACCCCGCCCCCATGCGCGTCTCC AAGTCTGCATACGCTCCTGTCCATCGTCTCTTCTGGGCGTCTGCTCGGCTCCTGG CGCGCGCTGGCCGCTGCCCTGGCTGCCGCGGGCGGGCTCAGCAAGTCGTGGTGGCC TCCTGGCGACGTGGCTGGGCGCGTCCATGCTCCGCTCTTGCTCTGA <u>gtaccacgcccgcgcgg</u> <u>cgcacctgacctgttctctcagggcgccgtgtctgccttcggaacaagccctcgagcatgcgtgcatgatcgtctctgcccgcgcgc</u> <u>gcggtttgtcccctcgcggcgccccggcgcgggcgccattgaaattgttcaaacccacctgacagattgagggccagcgagga</u> <u>agcggttgagatggaggtacaggagtcagtaactgaaagttttatgataactaacaacaaagggtcgtttctgccagcgcaatgacaagaa</u> <u>caagattccacatttcgtgtagaggttgccatcgatgtgagcggcgccgcggacccgacaaacccctacgacgtggtgaagaaa</u> <u>acgtgcgccgcaactgtccctgtagcctgaagaccagcaggagacgatcggaagcatcacagcacagcccgcccgctctcgaacagag</u> <u>cgcggcgcccgcatcgatacctgcaggacagcgccatgccacgccccttgatggcttcaagtacgattacgggtgttgattgtgtgttg</u> <u>ttcgtagtgtcatggttttagaataatacacttgatttctgtcaccgcaatctcggtgttcgcagggttaacccatttcggagct</u> <u>caggtcagccgcgaatgaccagccgctacttcaaggacttgacgacaacccgaggtgagctatgtttaggacttgattggaaat</u> <u>tgctgtcgacgatattcgctccgcgacagacccaagcaaatgtcaagtgcgttcgatttcgctccgaggtcgatgtgtgat</u> <u>cgctggcgccggatccgggtctgtctgccttacgagctgaccaagcaccctgacgtccgggtacgagctgagattcgattag</u> <u>acataaattgaagattaaacccgtagaaaaatttgatggctcgcaaaactgtgctcgattgcaagaaattgatcgtccctcactccgca</u> <u>ggctgccatcatcgagcaggcggtgtctccggcgccggtggtgggggacagctgttctcgccatgtgtgtacgtagaag</u>

SEQ ID NO:	Nucleotide Sequence
	gatgaatttcagctgggttttcgtgcacagctgtttgtgcatgattgtttcagactattgtgaatgttttagattcttaggatgcatgatgtgtctgcatgcgact

[0337] The construct can be written as

5'Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*GvirLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *GvirLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *GvirLPAAT2*. The initiator ATG and terminator TGA codons of the *GvirLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0338] **Table 93** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1219. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 93: Screen of primary transformants of CHK22 with pCHK1219.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1219-1	0.04	1.47	28.49	3.05	57.19	7.66	0.53	0.22	0.00	3.5
pCHK1219-2	0.04	1.40	28.61	2.69	57.45	7.61	0.53	0.21	0.00	3.6
pCHK1219-3	0.04	1.40	28.80	2.77	57.34	7.49	0.52	0.21	0.00	3.6
pCHK1219-4	0.06	2.05	29.69	2.95	54.85	8.02	0.73	0.21	0.00	3.2
pCHK1219-5	0.04	1.45	28.74	2.88	56.88	7.84	0.53	0.22	0.00	3.4

pCHK1219-6	0.04	1.42	29.01	3.19	57.37	6.86	0.47	0.26	0.00	2.3
pCHK1219-7	0.04	1.34	27.57	2.54	58.15	8.13	0.54	0.20	0.00	3.6
pCHK1219-8	0.04	1.49	28.95	3.11	56.65	7.65	0.53	0.23	0.00	3.3
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 45: Expressing *Volvox ferrisii* (*VferLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0339] *VferLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct, pCHK1220, contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 94**.

Table 94: Integrative sequences for the transformation of *P. moriformis* with pCHK1220 encoding *VferLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
75	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgcctccttggcagcgctctacacgtcgagtacgcc atgagggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgcgacgtgcagca gtccctctgcagcagatgagcgtgactttggcaattcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgtccagatcgacagatagcgaccatgtgtgctgctgaaaatctggattccgaattcgacc ctggcgctccatccatgaacagatggcgacactgttacaattctgtcacccatcgcatggagcagggtccacttagattcccgatc accacgcacatctcgtaaatagtcattcgttctgttcgatcaatcgaagtgtgcatggatcttggttgacgatgcggatg ggtttgcgcgctggctgcagggtctgccaaggcaagtaaccagctcctctccccgacaatactctgcaggcgaagccgggtca cttgccttccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtaacgtctgtctctgaccgtt ccccagcctctggcgctccctgccccgccaccagcccacgcgcggcgagtcgtgccaaggctgtctcggaCTTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCATTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtgcaggccttctgttctgtgtgcccgttcgccccaagatcagcg ctccatgacgaacgagacgtccgacgccccctggtgcaacttcaccccaacaagggtggtatgaacgaccccaacggcctgtggtgta cgacgagaaggacccaagtggcacctgtacttccagtacaacccgaacgacaccgtctggggacgccctgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcttctccggctccatgg tggtggactacaacaacacctccggcttcttcaacgacacccatcgacccgcgacgctgcgtggccatctggacctacaacaccccg gagtcgaggagcagtagatctctacagcctggacggcggtacaccttcaccgagtagaccagaagaaccccgctgctggccgccaact ccacccagttccgagaccgaaggctctgtgtacgagccctccgaagtgatcatgaccgcgccaagtcaccaggtactacaagat cgagatctactctccgacgacctgaagtctggaagctggagtcgcgttcgccaacgagggtctctcggtaccagtagcagtgccc cggcctgatcgaggtccccaccgagcaggaccccaagtcctactgggtgatgttcatctccatcaaccccgcgccccggcgccg ggctccttcaaccagtacttctgctggcagcttcaacggcaccacacttcaggccttcgacaaccagtcccgtgtgtgacttcggcaag

SEQ ID NO:	Nucleotide Sequence
	gactactacgccctgcagaccttctcaacaccgacccgacctacgggagcgccttgggcatcgcgtgggctccaactgggagtactc cgcttcgtgcccaccaacccctggcgctcctccatgtccctgtgcgcaagttctccctcaacaccgagtaggagccaacccggagac ggagctgatcaacctgaaggccgagccgatectgaacatcagcaacgccggccctggagccgggtcgcaccaacaccacgttgac gaaggccaacagctacaacgtcgaacctgtccaacagcaccggcaccctggagttcgaagctggtgtacgccgtcaacaccaccagac gatctccaagtccgtgttcgaggacctctccctctgttcaaggccctggaggaccccgaggagtacacctcccatgggttcgaggtgtcc gcgtcctccttcttctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttccaacccgcatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtcctactacaaggtgtacggcttggaccagaacatcctggagctgtacttcaacgacg gcgacgtcgtgtccaccaacacctacttcatgaccaccgggaacgccctgggctccgtgaacatgacgacgggggtggacaacctgttc tacatcgacaagttccaggtgcgcgaggtcaagtgaactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCGCGCGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAAGATTCCACATTTCCTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcggatagatcgacacactctggacgtggctgtgtatggac tgttccgccacactgtcgtccttgacctgtgaatatccctgccgttttatcaaacagccctcagtggtgttgcattgtgtgtacgcgcttttgcg agttgtactgtcgtgtgtatgttgcgaataccacccccagatccccctcctcgtttcatatcgcttgcacccaaccgcaacttatctacgc tgtcctgtatccctcagcgtcgtcctcgtcctcgtcactgccctcgcacagccttggttgggtccgctgtattctcgtgtaactgcaacc tgtaaacagcactgcaatgtgatgcacgggaagtagtgggtagggaacacaaatggacntaggaataacaatcaITCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAACCCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGCGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCCAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCGGGATCA TCGACCCCGTGGGCGGGCCCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCAC AATGTCTTCATCAGCAAGGCCGTCGGTCTGCCAGCTTCTGTTCTCCGTGTTCTGTGT TCTACTGGAGCCTGCCCATCTTCGCGCTGACGTACCGCATCCGCTTCGCGTCGAAGGGC AAGCGCAACGACATGCTGGAGTGGGCTAAGGCCCTGGTCCGCTTTTTTCGGCGTGAAGG TGCTCACGCAGGGCGAGCACTCGCTGTACCGCGGGGGCCCGTGCTCTACCTCTGCAA CCATCGGAGCTGGGCGGACTTTTTTCATCGACGCCTACCTGACCGAGGGCCGAGCTGCG CTGATGAGCCGATGGCTCGTCTACTTCGTGTTCCCCATCTTCTGTACCTCCGTGATGGT CCTGCGCGGGATCGTCCTTCAAGCGTGGGACCATCGCCGATAAGGAGGCCCTTCAAC GCCTGGCTCGACGCCAAGATGAAGTCTCGACCATTCGGGGGCTCCTGGTCTACCCCG

SEQ ID NO:	Nucleotide Sequence
	AGGGGCACCGCAGCCTCAAGCGCACGTCTCTGCCCCCTCAAGCGGGGCATGCTCCATTT TGCGTTCTCGCGCGGCCTGCCCCGTCCAACTCGTATGTGTGCTGGCAAAGGAGGAGGTC CTGTCTGAGAAGCGCATGGCCGTGCGCTTCGGTCGGACCCTCGTCGCCACGTACTCCG AGGTCTCTAAGCCCGGCAACTACCCGACGTTTCGACGCCTTCTTTTCCGGCATCCAGACG GCCTGGGATAGCTGCTGGCAGGCGGCTTACAAGCAGCCTCCCCAGGGTGCCGCGCCCC TGCCCCATGGTGAACACGACCGAGTACGACTATCCCCCGCGCATGCGCCGCTGCAACT GATCGTGACCCCTCCTGTCGATCGTCGTGCTGGCCGCGGTGCTCGCGGGCAGCGTCTTG GTCACCGTGGTCGCCGCTGCCGCGGTGGGCCCCATGCCAGCGGGCGTGGTCTGCGCCG TGCTCGCTAGCTGGCTCGGGGGCAGCATGCTGCGTTCTTTCCTCTGA <u>gtaccacgcccgcgcgcg</u> <u>gcacctgacctgttctcgcgggcgcctgttctgccttgcgaaacaagccctggagcatgcgtgcatgacgtctctggcgccccgcgcg</u> <u>cggtttctcgcctcgcgggcgcggcgccccggcgcgggcgcgccattgaaattgttgcaaacccacctgacagattaggcgccagcgaggaa</u> <u>ggcgttgagatggaggtacaggagtcagtaactgaaattttatgataactaacaacaagggtcgttctggccagcgaatgacaagaac</u> <u>aagattccacatttccgtgtagaggcttccatcgaatgtgagcggcgcgccgcggacccgacaaaaccttacgacgtggttaagaaaaa</u> <u>cgtgcggcgccactgtccctgtagcctgaagaccagcaggagacgacggaagcatcacagcacaggccgccccgcgtctcgaacagagc</u> <u>gcggcgccgcgcagatcctgcaggacagcgccatgccagcccttgcgtgcttcaagtagcattacggtgttggtgtgtgtgtgt</u> gcgtagtgtgcatggttagaataatacacttgatttcttgcacggcaatctcggttgcgcagggtcaacccatttcggagtctc aggtcagccgcgcaatgaccagccgctacttcaaggacttgcacgacaacgccagggtgagctatgttaggacttgattggaatt gtcgtcagcgcataatcgcgtccgcgacagcacccaagcaaatgtcaagtgcgttcgatttgcgtccgaggtgcaggtgtgtgac gtcggcgccgcatccgcggctgtctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgattaga cataaattgaagattaaacccgtagaaaaattgatggtcgcgaaactgtgctcgattgcaagaaattgatgctctccactccgcag gtcgccatcatcgagcaggcggtgtctccggcgcgccgctggctggggggacagctgtctcggccatgtgtgtacgtagaagg atgaatttcagctggtttcgttgacagctgtttgtgcatgattgtttcagactattgttgatgttttagatttcttaggatgcatgttt gtctgcatgcgact

[0340] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:*VferLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *VferLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *VferLPAAT2*. The initiator ATG and terminator TGA codons of the *VferLPAAT2*

are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0341] **Table 95** shows the fatty acid/TAG profiles of eight primary transformants of CHK22 transformed with pCHK1220. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 95: Screen of primary transformants of CHK22 with pCHK1220.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1220-1	0.03	1.20	27.20	2.94	59.80	6.56	0.49	0.27	0.00	1.57
pCHK1220-2	0.03	1.20	26.82	3.00	60.34	6.37	0.47	0.27	0.00	1.59
pCHK1220-3	0.03	1.20	26.72	2.99	60.48	6.36	0.47	0.26	0.00	1.51
pCHK1220-4	0.03	1.29	27.67	2.76	58.35	7.65	0.48	0.24	0.00	3.69
pCHK1220-5	0.03	1.21	27.13	2.98	59.91	6.48	0.48	0.27	0.00	1.48
pCHK1220-6	0.04	1.29	27.77	2.76	58.46	7.42	0.48	0.24	0.00	3.3
pCHK1220-7	0.03	1.24	27.68	2.58	58.86	7.34	0.48	0.23	0.00	3.8
pCHK1220-8	0.03	1.20	27.20	2.97	59.80	6.52	0.49	0.27	0.00	1.49
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 46: Expressing *Vitreochlamys aulata* (*VaulLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0342] *VaulLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1222 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 96**.

Table 96: Integrative sequences for the transformation of *P. moriformis* with pCHK1222 encoding *VaulLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
76	ccctcaactgcgacgtgggaaccttctccgggcaggcgatgtgcgtgggttgcctccttggcagcgctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgggtggcactctgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgtgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgctccagatgcacagatagcgaccatgtgtgctgcgtctgaaaatctggattccgaattcgacc

SEQ ID NO:	Nucleotide Sequence
	ctggcgctccatccatgcaacagatggcgacacttgttacaattctgtcaccatcgcatggagcaggccacttagattcccgatc acccagcacatctcgctaatagtcattcgttctgtcttcgatcaatctcaagtgagtgatggatcttggttgacgatgcggtatg gggttgcgccgtggctgcagggtctgccaaggcaagtaaccagctcctctccccgacaatactctcgaggcaaaagccggtca cttgccttccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatgggtcacgctcgtgtcctgaccgtt cccagcctctggcgctccctgccccgccaccagccccagccgcggcgagctcgtgccaaggctgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTTTCA GTCACAACCCGCAAACTGTACAATGctgtcagggccttctgttctgtgtgcccgttcgccccaagatcagcg cctccatgacgaacgagacgtccgacggccccctggtgcacttcaccccaacaaggctggatgaacgaccccaacggcctgtgga cgacgagaaggacgccaagtggcactgtacttccagtacaacccgaacgacaccgtctggggacgcccttgttggggccacgcc acgtccgacgacctgaccaactgggaggaccagccccatcgccatcgccccgaagcgcaacgactccggcgcccttccggctccatgg tgggtgactacaacaacacctcggtcttcaacgacaccatcgacccgcgcagcgctgcgtggccatctggacctacaacacccccg gagtccgaggagcagtacatctctacagcctggacggcggtacaccttcaccgagtaccagaagaacccccgtgtggccgcaact ccaccagttccgcgacccgaaggtcttctgttacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagctctggaagctggagtcgcgttcgccaacgagggttctccgctaccagtaacgagtcccc cggcctgatcgagggtccccaccgagcaggacccccagcaagtctactgggtgatgttcatctccatcaacccccgcgccccggccggc ggctccttcaaccagtactctgcggcagcttcaacggcaccacactcgaggccttcgacaaccagtcgccgtgtgggacttcggcaag gactactacgccctgcagaccttctcaacaccgacccgacctacgggagcgccctgggcatcgctgggctccaactgggagtactc cgcttcgtgccccaccaacccctggcgctcctccatgtccctgtgcgcaagtctccctcaacaccgagtaccaggccaacccggagac ggagctgatcaacctgaagccgagccgatectgaacatcagcaacgccggccccctggagccggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcaccctggagttcgagctggtgtacgccgtcaacaccaccagac gatctcaagtcgtgttcgggacctctcctctgttcaaggcgctggagacccccgaggtacacctccgcatgggttcgaggtgtcc gcgtcctccttctctggaccgcgggaacagcaaggtgaagttctgtaaggagaacccctacttcaacacccgatgagcgtgaacaa ccagcccttcaagagcgagaacgacctgtctactacaaggtgtacggcttctgtggaccagaacatcctggagctgtacttcaacgacg gcgacgtcgtgtccaccaacacacttctatgaccaccgggaacgccctgggctccgtgaacatgacgacgggggtggacaacctgtt tacatcgacaagttccaggtgcgcgaggtcaagtactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCCTGGAGCATGCGTGATGATCG TCTCTGGCGCCCCCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcggatagatcgacacactctggacgctggctgtgtatggac tgttgcggccacacttgcgtccttgacctgtgaatatcctgcgctttatcaaacagcctcagtggtttgatcttgtgtgtacgcgcttttgcg

SEQ ID NO:	Nucleotide Sequence
	<u>agttgctagctgcttgctatgttgcgaatacaccacccagcatcccttcctcgttcatatcgcttgcacccaaccgcaacttatctacgc</u> <u>tgctcgtctatccctcagcgctgctcctgctcctgctcactgccctcgacagccttggttgggctccgctgtattctcctggctactgcaacc</u> <u>tgtaaacagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatca</u><u>TTCGCCG</u> <u>CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTTATGCCAGACTGGGTCCC</u> <u>AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTCTGGAGGCGGGGCACCCCCGCTCTC</u> <u>TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAACCCTGCCAGAAACAATTG</u> <u>AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC</u> <u>TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTCTGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TCGACCCCCGTGGGCCGGGCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC</u> <u>A</u><u>ATGGGCGCCGCGTCGAAGGTGCTGGGCCTGCCCTCCTTCTCTTCTCCGTCTTCGTCC</u> <u>TGTACTGGTCTCTGCCCATCTTCGCGGTGCTCTACCGCATCCGATTGCCAAGATGATC</u> <u>GGCAAGCGCAACGACGTGCTGGACTGGGCCAAGTCTCTCGTGCGGTTTTTCGGCATCA</u> <u>CCGTCTGCGCCAGGGCCAAGAGCAACTGTACACGGGTGGCCCTGCCTGTACCTGTG</u> <u>CAACCACCGCTCGTGGGCCGATTTCTTTCTGGACGTGTACCTGACCGAGGGCCACGCG</u> <u>GCCGTATGTCTGCGCTGGCTGGTCTACTTCGTCTTCCCGGTCTTCTGCACGTCTGCCT</u> <u>GATCCTCAAGACGATTGTCTCTTCAAGCGCGGCACCATCCGCGACAAGGAGGGCTTTA</u> <u>ACCGCTGGCTGGACGCCGTCTGGCCCGCTCGAACGTGCCTGGCCTCCTGGTGTTC</u> <u>GGAGGGCCACCGCAGCCTGCGTCCCGCCTCGCTGCCACTGAAGCGGGGCATGCTGCAT</u> <u>TACGCCTACGGCCGGAAGCTGGCGATCCAGATGGTGGTCACGCGTGGCAAGGAGGAGG</u> <u>TGATGTCCGAGAAGACCATGGCCGTCCGCTTCGGCCGCACGCTGGTCACGACCTACTC</u> <u>CAAGGTCTGCGCCCGGCCGACTACCCTGACTTTGAGGCGTCTTTGCGGACCTCCAAGT</u> <u>CCACCTGGGACGGCTGCTGGGAGGCCGCGTACCGGAAGCCTCGGGAGGACGCCGTGC</u> <u>GGCACTCTCTGGGCGCGGCCCGGCTTTCACTACCCCTTTGCGACGCGTGTGGCGCA</u> <u>ACTGGGCGCCACCCTGGGGTCCATCGCCCTGTTGCGCCTGGTCTGGTGGGCTGCTGG</u> <u>CGTGGCCTGGCCGCTGCCCTGGCGGCCACGGGCGCCGCGCAGCAAGCGGTGCGCCG</u> <u>CTCCTGGCCGTCTGGGTGGGTGGCTCCATGCTCCGTTTCGTTCTGTGA</u><u>gtaccagcccgccg</u> <u>gcgcacctgacctgttctctcgaggcgccctgttctgccttcggaacaagccccggagcatgcgtcatgatcgtctctggcgccccgccg</u> <u>cgcggtttgtcgccctcgcgccgccccgccgcggggcgccattgaaattgttgaacccccacctgacagattgagggccagcgagg</u> <u>aagcgcttgatgaggtacagagtaagtaactgaaagtgtttatgataactaacaacaagggtcgttctgcccagcgaatgacaaga</u> <u>acaagattccacatttccgtgtagagccttgccatcgaatgtgagcgggcgccgcccggacccgacaaaaccttacgacgtgtaagaaa</u> <u>aacgtggcgccgactgtccctgtagcctgaagaccagcaggagacgacggaagcatcacagcacagccggcccgctctcgaacaga</u> <u>gcggcgccggccgcatcgatacctgcaggacagcgccatgccacgccctttagggcttcaagtacgattacgggtgttgattgtgttt</u> <u>gttgctagtgtgcatggttagaataatacacttgatttcttctcacggcaatctcggttctcgcaggttcaacccatttcggagt</u> <u>ctcaggtcagccgcgaatgaccagccgctacttcaaggactgcacgacaacccgaggtgagctatgtttaggacttgattggaa</u> <u>attgtcgtcagcgaatttcgctccgcgacagacccaagcaaatgtcaagtgcgttccgatttgcgtccgcaggtcgatgttgtg</u>

SEQ ID NO:	Nucleotide Sequence
	atcgtcggcgccggatccgcccgtctgtcctgcgttacgagctgaccaagcacctgacgtccgggtacgcgagctgagattcgatt agacataaattgaagattaaccgtagaaaaatttgatggcgcgaaactgtgctcgattgcaagaaattgatcgctccactccg caggtcgccatcatcgagcagggcgttgcctccggcgccgcctggctggggggacagctgttctcgccatgtgtgtacgtaga aggatgaatttcagctggttttcgtgcacagctgtttgtcatgattgtttcagactattgtgaatgttttagatttcttaggatgatg atttgtctgcatcgact

[0343] The construct can be written as 5'Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*VaulLPAAT2*:PmPGH::3'Thia4. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *VaulLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *VaulLPAAT2*. The initiator ATG and terminator TGA codons of the *VaulLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0344] Table 97 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1222. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 97: Screen of primary transformants of CHK22 with pCHK1222.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1222-1	0.04	1.43	28.52	2.75	57.09	7.99	0.52	0.19	0.00	3.5
pCHK1222-2	0.04	1.40	28.30	2.70	56.85	8.52	0.52	0.19	0.00	3.6

pCHK1222-3	0.04	1.35	27.70	2.96	59.21	6.63	0.48	0.23	0.00	1.5
pCHK1222-4	0.03	1.13	28.97	1.82	57.29	8.40	0.47	0.14	0.03	4.6
pCHK1222-5	0.04	1.45	28.48	3.17	57.53	7.29	0.50	0.22	0.00	3.1
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 47: Expressing *Chlamydomonas* sp. CCAC2762_B (*Ch*_CCAC2762_LPAAT2) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0345] *Ch*_CCAC2762_LPAAT2 was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1224 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 98**.

Table 98: Integrative sequences for the transformation of *P. moriformis* with pCHK1224 encoding *Ch*_CCAC2762_LPAAT2.

SEQ ID NO:	Nucleotide Sequence
77	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgcctccttggcagcgctctacaccgtcgagtacgcc atgagggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgcgacgtgcagca gtccctctgcagcagatgagcgtgactttggcaattcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgtccagatcgacagatagcgaccatgttgcgtcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgaacagatggcgacactgttacaattcctgtcacccatcgcatggagcagggtccacttagattcccgatc accacgcacatctcgtaatagtcattcgttctgttcgatcaatcgaagtgtgcatggatcttggttgacgatgcggtatg ggtttgcgcgctggctgcagggtctgccaaggcaagtaaccagctcctctccccgacaatactctcgaggcaaaagccggtca cttgccttccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtaacgtcgtgtcctgaccgtt ccccagcctctggcgctccctgccccgccaccagcccagccgcggcgagtcgctgccaaggctgtctcggaCTTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACCTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtgcaggccttctgttctgtgtggccggttcgccccaagatcagcg ctccatgacgaacgagacgtccgacgccccctggtgcacttcaccccaacaagggtggaatgaacgacccaacggcctgtgga cgacgagaaggacccaagtggcacctgtacttcagtagaaccggaacgacacccgtctggggacgacctgtgtgggcccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcttctccggtccatgg tggtggactacaacaacacctcggcttcttcaacgacacccatcgacccgcgacgctgcgtggccatctggacctacaacaccccg gagtcgaggagcagtagatctctacagcctggacggcggtacaccttcaccgagtagcagaagaacccccgtgtggtggcccaact ccacccagtcccgacccgaaggctctgtgtacgagccctccagaagtggatcatgaccgaggaagtcacaggactacaagat cgagatctactctccgacgacctgaagtctggaagctggagtcgcgttcgccaacgagggtctcctcggtaccagtagcagtgccc cggcctgatcgaggtccccaccgagcaggaccccaagtcctactgggtgatgttcatctccatcaacccccggcgccccggcgcc ggctccttcaaccagtacttctgtcgagcttcaacggcaccacacttcagggccttcgacaaccagtcccgtgtgtgacttcggcaag

SEQ ID NO:	Nucleotide Sequence
	gactactacgccctgcagaccttctcaacaccgacccgacctacgggagcgccttgggcatcgcgtgggctccaactgggagtactc cgcttcgtgcccaccaacccctggcgctcctccatgtccctgtgcgcaagttctccctcaacaccgagtaggagccaacccggagac ggagctgatcaacctgaaggccgagccgatectgaacatcagcaacgccggccctggagccgggtcgcaccaacaccacgttgac gaaggccaacagctacaacgtcgaacctgtccaacagcaccggcacccctggagttcgagctgggtacgccgtcaacaccaccagac gatctccaagtccgtgttcgaggacctctccctctggttcaaggccctggaggaccccgaggagtacccctccatgggttcgaggtgtcc gcgtcctctcttctcctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttccaacccgcatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtcctactacaaggtgtacggcttggtagcagaacatcctggagctgtacttcaacgacg gcgacgtcgtgtccaccaacacctacttcatgaccaccgggaacgccctgggctcctggaacatgacgacgggggtggacaacctgttc tacatcgacaagtccaggtgcgcgaggtcaagtgaactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCGCGCGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAAGATTCCACATTTCCTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcggatagatcgcacacactctggacgtggctgtgtatggac tgttccgccacactgtcgtccttgacctgtgaatccctgccgttttatcaaacagccctcagtggtgttgcattgtgtgtacgcgcttttgcg agttgtcagctgtgtgtctatttgcgaalaccacccccagatccccctcctcgtttcatatcgcttgcacccaaccgcaacttatctacgc tgtcctgtatccctcagcgtcgtcctcgtcctcgtcactgccctcgcacagccttgggttgggtccgcctgtattctcgtgtaactgcaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtgggtagggaacacaaatggacntaggaataacaatcaITCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAAACCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGCGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCCAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCGGGATCA TCGACCCCGTGGGCCGGGCCCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCAC AATGCGCCAGCTCAAGTCCCTGATCCAACATGCCCTACTTCCTGTTTTGTGTCTTCGTGTT CTACTGGTCCTTCCCCATCGGCTGCCTCACCATCACATCCACTTCCTGAAGTTTTCCGG CAAGCGCAACGACATGCACACCTGGTCTGAGACCATGTGCTCTTTCTTTGGCATCCGCT TCGTGAAGTACGGTGAGGAGAAGCTCAGCAAGGGTCCCGCAACATCCTCTACCTGTCC AACCACCGCTCGTGGGCCGACTTTTTCGTCGATGTCTACCTGACCGAGGGCAACGCCGC GATGCTCAGCCGATGCTGGTCTTCTTTGTCTTTCCATCTTCATGACCTCGGTCTGTT CCTGAAGGGCATTATCCTGTTCAAGCGCGGCACGATCGCCGACAAGGAGAAGTTCAAC GCCTGGCTGGATGTGATGCTGGAACGATCGCCCATCCCCGGAATGCTGGTCTACCCCG

SEQ ID NO:	Nucleotide Sequence
	AGGGCCATCGGTCGACGCGGCCCCACAGCCTCCCGCTGAAGCGCGGCATGCTGTACTA CGCCCACAGCCGGGGCATGCCCCTGCAGATCATTATCTCGAACGGCAAGGAGGGCGTC ATGTCGGAGAAGCAGCGCCGAGCGGGCTTTGGCAAGACGGTCGTGGTGGCTTCTCTT CGGTGATTGACACCGCGGCCTACAAGGGCAACGTCGAGGGCTTTATGGCGCGAGTGCA GGAGGAGTGGAACAAGCTGTGGGGTCAGGTCTACTCCGCCGACCCGGCGGGCCTGCCC CCTCTGCGCCAAGATCCCCGATGTCTGGACTACGCGGCCAGCATGCGAACGCAGCAAC TGGCCATCGCCGTCTTCTCCATGTTGCGCCCTGGCTGGCGTGCTGTGGGTGACGTGGCG CATGTTGCGTGGCTGACCGCCCTGGCCGGGCCCCCTGCAACACGCCCTGGCCGTGCTC CTGCTCACCTGGTTCGCTGCGTCCATTATCGCGGGCGTGATGGGCCCCGTCGCTCCCGAC CCACTCTTCCTCGCTCCAGAAATCCCCCTTCTCTGAAGGCCGACAAGAGCGTGTCGGCCG CTGCCGCTGCCGCTGCCGCGGCCTCCGGCGACGGGAGCCTCCACGAGGGCCCCTAGCGG CAAGGCCCCGGCTGCCGCTGCCACCCAGAACCACAACCACCAACTGA<u>gtaccacgccccgc</u> <u>cggcgacactgacctgttctctcagggcgccgtgttctgccttgcgaacaagccccctggagcatcgctgcatgatcgtctctgcgccccgc</u> <u>cgcgcggttctgcctcgcggcgccccggcgcgggggcgccattgaaattgttcaaacccacctgacagattgaggccagga</u> <u>ggaaggcgttgagatggaggtagaggtacaggagtaagtaactgaaagttttatgataactaacaacaagggtcgttctgcccagcgaatgacaa</u> <u>gaacaagattccacatttccgtgtagaggctgccatcgaatgtgagcggcgccggcgacccgacaaaaccttacgacgtggtgaaga</u> <u>aaaacgtgcggcgactgtccctgtagcctgaagaccagcaggagacgatcggagcatcacagcagagccggccccgcgtctcgaaca</u> <u>gagcgcgccggcgccatcgatacctgcaggacagcgccatgccacgccccttgcgtggttcaagtacgattacgggtgtgtgtgtgtg</u> <u>ttgtgtgctagtgcatggtttagaataatacattgatttctgtcacggcaatctcggtgtccgcagggtcaacccatttcgga</u> <u>gtctcaggtcagccgcgaatgaccagccgctactcaaggactgcacgacaacgccgaggtgagctatgtttaggacttgattgga</u> <u>aattgtcgtcagcgaatttcgcgtccgcgacgacccaagcaaatgtcaagtgcgttcgatttgcgtccgaggtcagtgattgt</u> <u>gatcgtcgccgcccgatccggctgtctgcgttacgagctgaccaagcaccctgacgtccgggtacgcgagctgagattcgat</u> <u>tagacataaattgaagattaaaccgtagaaaaattgatggtcgcaaaactgtctcgattgcaagaaattgatcgtccctcactcc</u> <u>gcaggtgccatcatcgagcaggcggtgtctccggcgccgctggctggggggacagctgttctcgccatgtgtgtacgtag</u> <u>aaggatgaattcagctggtttcggtgcacagctgtttgtcatgattgtttcagactattgtgaatgttttagatttcttagatgcat</u> <u>gattgtgtcgtacgcact</u>

[0346] The construct can be written as

5'Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*Ch_CCAC2762_LPAAT2*:PmPGH::3'Thi
a4. Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from
CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C.*
reinhardtii β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring
the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator
ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding
region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables
amplification of the *Ch_CCAC2762_LPAAT2* gene, is indicated by uppercase underlined text

followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. A PmG3PDH promoter from *P. moriformis*, indicated by the boxed italicized text, drives the expression of the *Ch_CCAC2762_LPAAT2*. The initiator ATG and terminator TGA codons of the *Ch_CCAC2762_LPAAT2* are indicated in uppercase, bold italics, while the remainder of the gene is indicated in bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0347] Table 99 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1224. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 99: Screen of primary transformants of CHK22 with pCHK1224.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1224-1	0.03	1.25	29.39	2.29	57.45	7.55	0.48	0.21	0.00	4.59
pCHK1224-2	0.03	1.06	25.88	3.15	61.04	6.93	0.47	0.27	0.00	1.65
pCHK1224-3	0.03	1.18	26.12	3.21	60.96	6.52	0.51	0.29	0.00	1.47
pCHK1224-4	0.05	2.02	27.54	3.09	57.62	7.54	0.63	0.23	0.00	3.83
pCHK1224-5	0.03	1.36	27.00	3.18	59.02	7.34	0.59	0.28	0.00	1.83
pCHK1224-6	0.03	1.23	26.13	3.37	60.46	6.86	0.52	0.30	0.08	1.91
pCHK1224-7	0.03	1.23	28.61	2.97	55.28	9.69	1.33	0.39	0.00	2.23
pCHK1224-8	0.03	1.22	28.38	2.94	58.67	6.84	0.46	0.24	0.05	3.12
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 48: Expressing *Dunaliella salina* (*DsalLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDG promoter.

[0348] *DsalLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1225 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 100**.

Table 100: Integrative sequences for the transformation of *P. moriformis* with pCHK1225 encoding *DsalLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
78	cctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggttgctccttggcacggctctacaccgtcagtagcc atgaggcggtgatggtctgtcgttgccacttcgtccagagacggcaagtcgtccatcctctcgtgtgtggcgacgtcgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatcatttttcttaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgtccagatcgcacagatagcgacctgttgctcgctctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttggtacaattctctgtcacccatcggcattggagcagggtccacttagattcccgat accacgcacatctcgtaaatgatacttcgtctgtcttcgatcaatctcaagtgagtgtgcatggatcttgggtgacgatcggtatg ggtttgcgcgctggctgcagggtctgccaaggcaagctaaccagctcctctccccgacaatactctgcaggcaaagccgggtca cttgccttccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgtctgtctcgaccgtt ccccagcctctggcgctccccgcccaccagccccagccgcggcgagtcgctgccaaggctgtctcggaCTTCTCTTGC GCTATGACACTTCCAGCAAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAAACACCTAGATCACTACCACCTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgctgcaggcccttctgttctgctggccggcttcgcccaagatcagcg cctccatgacgaacgagacgtccgaccgccccctgggtgcacttcaccccccaacaagggtggatgaacgaccccaacggcctgtggt cgacgagaaggacccaagtggcacctgtacttcagtagacaacccgaacgacaccgctctgggggacgcccttgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcccttctccggtccatgg tggtggactacaacaacacctccggcttcttaacgacaccatcgaccgcgccagcgctgcgtggccatctggacctacaacaccccg gagtcggaggagcagtagatctctacagcctggacggcggtacaccttcaccgagtaccagaagaaccccgctgctggccccaact ccaccagttccgcgacccgaaggcttcttggtacgagccctccagaagtggatcatgaccggcgcaagtccaggactacaagat cgagatctactctccgacgacctgaagtctggaagtggagtcgcgttcgccaacgagggtctctcggtaccagtagcagtgccc cggcctgatcgagggtcccccagcaggacccagcaagtctactgggtgatgttcattctcatcaaccccgccgccccggccggc ggctccttcaaccagtacttctgcgcagcttcaacggcaccccaacttcgaggcttcgacaaccagttcccgctggtggacttcggcaag gactactacgcccctgcagaccttcttaacaccgacccgacctacgggagcgccctgggcatcgctgggctccaactgggagtactc cgcttcgtgcccaccaaacccctggcgtctctccatgtccctctgtgcgaagtctccctcaaacccgagtaccaggccaacccggagac ggagctgatcaacctgaaggccgagccgacgtgaacatcagcaacgccggccccctggagccgggttcgccaccaacaccacgttgac gaaggccaacagctiacaacgtcgacctgtccaacagcacccggcacccctggagttcgagctggtgtacgccgtcaacaccaccagac gatctccaagtccgtgttcgcgacctctccctctgttcaaggccctggaggaccccgaggagtacctccgatgggcttcgaggtgtcc gcgtctccttctcttgaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaccaaccgatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtctactacaaggtgtacggcttctgtggaccagaacatcctggagctgtacttcaacgacg gcgacgtcgtgtccaccaaacacttctcatgaccaccgggaacgccttgggtccgtgaacatgacgacgggggtgggacaacctgttc tatatcgacaaghtccaggtgcgaggttcaagtgactTAAGACGCCCCGCGCGGCACCTGACCTGTTCT CTCGAGGGCGCCTGTCTTCGCTTGCGAAAAAAGCCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCCGCGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGC GCATTGAAATTGTTGCAAAACCCACCTGACAGATTGAGGGCCCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA

SEQ ID NO:	Nucleotide Sequence
	GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAAACCGGTgcagcagcagctcggatagatcgacacactctggacgtggtcgtgtgatggac tgttgccgccacacttgctgacctgtaatatccctgccgtttatcaaacagcctcagtggtttgatcttgtgtacgcgttttg agttgtagctgcttgctattgcaataccacccccagcatcccttccctcgtttcatalcgttgcatcccaaccgcaacttatctacgc tgtctgtatccctcagcgtgctcctgctcctgctcactgccctcgcacagccttggttgggtccgcctgtattctcctggtactgcaacc tgtaaacagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatcaTTCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCAATTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAACCCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA TCGACCCCCGTGGGCCGGGCCCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACCC AATGGGCCAGTCCACCCTCCAGAAGCTCATTTCCCTGCCGACGTTCTTTCTCTCGATCT TCCTGATGTACTGGAGCCTGCCCCCTCTGCACCCCTGTGTGGCTACCTGCGCCTCCTGGAC TTTAAGTCCACGCGCAAGGACATGTACAACTGGTGCCGCTTCATGACGCGTCTCTTCCG TATCAAGGTCAAGAAGTACGGCTCCAAGTCGCTCCGCAAGGACGCGGGTCGGCATATC CTGTACCTGTGCAACCATCGTAGCTGGACGGACTTTTTCTCGACGCTTACCTGACCGA GGGCAACGCCTCCCTGATGTCCCGTTGGCTCGTGTGCGGTCTTTCCGGTCTTCATCG TGTCGGTGATCGTCTCCGGGGAGTCATCCTCTTCAAGCGCGGCACCATTCTGGACAAG GAGAAGTTTAATCGGTGGCTGGACTCGAAGATCCTGTGACCCCCGATCCCCGGCGTCCT CGTCTACCCCCGAGGGCCACCGCTCTCTGGCCGACAAGTCGCTGCCACTCAAGCGCGGC ATGCTCTACTACGCCCACTCCGAAAGTTCCAGTGCAAATCATTATCTCCCGCAACAA GGAGGACGTCCTGAGCGAGAAGACCATGAAGGCCCACTTCGGCGCCACGATTATCACG GGCTGCTCCGACCTGATCGAGTCTGACGGCAAGGACATCAAGGTCTTTCTGTCCGAGAT CCAGGCCGCGTGGGATGACCTGTGGGAGAAGGTGTACGCGGCCGACGCGACCCGCTCTG CCACCCCTGGAGCAGTCGAACATCCCCGAGTTCGACTACAGCATGGGGATGCGTGTCA AGCAGCTCCTGGTCGTGTGCAGCTCGGTGCTGGTGTCTTCTGGCGGCCATGGTCGTGTTT GGTAAGCTCTCTTGCTGATCTACGCCGCGATGGGTGACACCGAGCGCAAGGTCTCTGG TGACCTCATCTGTACGTCTGGTTTTCTGTGAGCATGCTCAACAGCCGGGCCCCGAGC GCCACGCAGTAGgtaccacgccgcgcgcgcacactgacctgttctctcagggcgccctgttctgacctcgaaacaagccctg gagcatgcgtgcatgatgtctctggcgccccgcgcgcggtttgtcgcctcgcggcgccccggcgcgccgagcattgaaattgtg caaacccacactgacagattgagggccaggcaggaagcgttgagatggaggtacaggagtaagtaactgaaagtttatgataactaa caacaagggtcgtttctggccagcgaatgacaagaacaagattccacatttccgttagaggcttgccatcgaatgtgagcggcgccgcgcg

SEQ ID NO:	Nucleotide Sequence
	<u>cggacccgacaaaacccttacgacgtgtaagaaaaacgtggcgggactgtccctgtagccctgaagaccagcaggagacgacgcggaag</u> <u>catcacagcacaggccggcccgctctcgaacagagcgcgggcgccgcacgcatacctgcaggacagcgccatgccacgcccttgat</u> <u>ggcttcaagtaacgattacgggtgttgattgtgtgtttgttcgtagtgcacgtgtttagaataatacacttgattcttctcacggcaat</u> <u>ctcggcttgcgcagggtcaaccccaatttcggagtcaggtcagccgcgaatgaccagccgctacttcaaggacttgacacgaaa</u> <u>cggcgaggtgagctatgttaggacttgattggaaattgtcgtcgacgcataatcgcgctccgcgacagcaccgaagcaaaatgtcaa</u> <u>gtcgttccgatttgcgtccgaggtcgatgtgtgatcgtcgccgcccggatccgcccgtctgtctgcgttacgagctgaccaagca</u> <u>ccctgacgtccgggtacgcgagctgagattcgattagacataaattgaagattaaaccgtagaaaaattgatggtcgcaaaactgt</u> <u>gtcgtgattgaagaaattgatcgtcctccactccgaggtcgccatcatcgagcaggcggtgtgtcccgccggcgccgtggtggg</u> <u>gggacagctgttctcgccatgtgtgtacgtagaaggatgaattcagctggtttcggtgcacagctgtttgtgcatgtttgttcaga</u> <u>ctattgtgaatgttttagatttcttaggatgcatgattgtctgcatgcgact</u>

[0349] The construct can be written as

5'Thia4::CrTUB2:Sc*SUC2*:PmPGH:CvNR:PmG3PDH:*Dsal**LPAAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *Dsal**LPAAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. A PmG3PDG promoter from *P. moriformis*, indicated by the boxed italicized text, drives the expression of the *Dsal**LPAAAT2*. The initiator ATG and terminator TAG codons of the *Dsal**LPAAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0350] **Table 101** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1225. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 101: Screen of primary transformants of CHK22 with pCHK1225.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1225-1	0.03	1.15	26.06	3.07	61.11	6.63	0.53	0.24	0.00	1.69
pCHK1225-2	0.03	1.20	26.00	3.28	61.09	6.56	0.52	0.26	0.00	1.49
pCHK1225-3	0.03	1.23	26.56	3.41	60.15	6.69	0.50	0.31	0.00	2.06
pCHK1225-4	0.03	1.28	25.40	3.28	61.54	6.55	0.54	0.25	0.00	1.56
pCHK1225-5	0.03	1.21	25.26	2.85	62.38	6.56	0.40	0.17	0.00	1.59
pCHK1225-6	0.06	1.72	19.85	3.23	66.00	7.47	0.44	0.20	0.00	1.57
pCHK1225-7	0.03	1.22	26.17	3.27	60.76	6.65	0.49	0.25	0.00	1.69
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 49: Expressing *Microglena* sp. *YARC* (*MyarcLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0351] *MyarcLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1227 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 102**.

Table 102: Integrative sequences for the transformation of *P. moriformis* with pCHK1227 encoding *MyarcLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
79	ccctcaactgcgacgctgggaacctctccgggcaggcgatgtgcgtgggtttgcctccttggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcggttgccactctgctccagagacggcaagtgcctcatccttgcgtgtgtggcgacgctcgagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttcttaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgctccagatcgacagatagcgacatgtgtgctgcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttggtacaattcctgtcaccatcgcatggagcaggctcacttagattcccgatc accacgcacatctcgtaatagtcattcgttctgtcttcgatcaatctcaagtgcgtgcatggatcttggtgacgatcgcggtatg ggtttgcgccgtggctgcagggtctgccaaggcaagctaaccagctcctctccccgacaatactctcgaggcaagccggtca cttgccttcagattgccaataaactcaattatggcctctgcatgccatccatgggtctgatgaatggtcacgctcgtgtctgaccgtt ccccagccttggtgctccctgccccgccaccagccacgcgcggcagtcgctgccaaggctgtctcggaCTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTTTCA GTCACAACCCGCAAACTGTACAATGctgtcgtcaggccttctgttctgtgtgcccgttcgcccaagatcagcg cctccatgacgaacgagacgtccgaccgccccctggtgcacttcaccccccaagggtgatgaacgaccccccaaggcctgtggtg

SEQ ID NO:	Nucleotide Sequence
	<i>c</i>gacgagaaggacccaagtggcacctgtacttccagtagacaacccgaacgacaccgtctggggagcccttgttctggggccacgcc <i>a</i>cgtccgacgacctgaccaactgggaggaccagcccatcgccatcgccccgaagcgcaacgactccggcgcttctccggctccatgg <i>t</i>ggtggactacaacaacacctecggcttcttaacgacaccatcgacccgcgccagcgtcgtggccatctggactacaacaccccg <i>g</i>agtcgaggaagcagtagatctcttacagcctggacggcgctacaccttaccgagtagcagaagaaccccggtgctggccgccaact <i>c</i>cacccagttccgcgacccgaaggtctctgtgtacgagccctccagaagtggtatcatgaccgcggccaagtccaggactacaagat <i>c</i>gagatctactctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggttctcggctaccagtagagtgccc <i>c</i>ggcctgatcgaggtccccaccgagcaggacccagcaagtcctactgggtgatgttcatctccatcaaccccggcgccccggccg <i>g</i>gctcctcaaccagtagtctcggcagcttcaacggcaccacacttcgaggttcgacaaccagtagccgcgtgggtgacttcggcaag <i>g</i>actactacgacctgagaccttcttaacaccgacccgacctacgggagcgccctgggcatcgctgggctccaactgggagtactc <i>c</i>gccttcgtgcccaccaacccctggcgctcctccatgtccctgtgcgcaagttctcctcaacaccgagtagcaggccaacccggagac <i>g</i>gagctgatcaacctgaagccgagccgactctgaacatcagcaacgcccggccctggagccggttcgccaccaacaccacgttgac <i>g</i>aagggccaacagctacaacgtcgacctgtccaacgacacggcacccctggagttcgagctgggtgtacgccgtcaacaccaccagac <i>g</i>atctccaagtccgtgttcgagacctctccctctgtgtcaaggcctggagaccccgaggagtagctccgcatgggttcgaggtgtcc <i>g</i>cgctcctcttctctgtgaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttccaacccgcatgagcgtgaacaa <i>c</i>cagccctcaagagcgagaacgacctgtctactacaaggtgtacgcttgcgtggaccagaacatctggagctgtacttcaacgacg <i>g</i>cgacgtcgtgtccaccaacacctattcatgaccaccgggaacgcccgtggctccgtgaacatgacgacgggggtggacaacctgttc <i>t</i>acatcgacaagttccaggtgcgcgaggtcaagtgtactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT <u>CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGATGATCG</u> <u>TCTCTGGCGCCCCGCGCGCGGTTTGTTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC</u> <u>GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCCAGGCAGGAAGGCGT</u> <u>TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA</u> <u>GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCTGTAGAGGC</u> <u>TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT</u> <u>AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG</u> <u>GAAGCATCACAGCACAACCGGTgcagcagcagctcgatagtagcagacactctggacgtgtgtgtgtgac</u> <i>gtgtgccgccacactgtgtgcttgccttgacctgtgaatatccctgccgttttataacacagcctcagtggtgtgtgtgtgtacgcgttttgcg</i> <i>agttgtagctgtgtgtgtatgttgcgaatacaccceccagcatcccttccctcgtttcatalcgttgcatcccaaccgcaacttatctacgc</i> <i>tgtctgtatccctcagcgtgtctctgtctctgtcactgcccctgcacagccttggttgggtccgctgtattctctgtgtagtgaacc</i> <i>tgtaaacagcactgcaatgtgtatgcacgggaagtagtggtggtgggaacacaaatggactaggaaataacaatca</i><u>TTCGCCG</u> <u>CTCAGCCTACACGTCTTCTCCGATACCTTCCCTCATTGCATTTATGCCAGACTGGGTCCC</u> <u>AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTCTGGAGGCGGGGACCCCCGCTCTC</u> <u>TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAACCCTGCCAGAAACAATTC</u> <u>AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC</u> <u>TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TCGACCCCGTGGGCCGGGCCCCCGGACCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTTACTCTTAATCTCGCTCTCCTTCATATACAACCACAAGTAATCAACTCACC</u>

SEQ ID NO:	Nucleotide Sequence
	<u>A</u>ATGTCGGCGGAGAAGCTGCTCCACGCTCCATCCTTTGTCATCACCTGCCTGGTGCTGT ACTGGTCCCTCCCTCTCTCCGTGCTGTTTCGGCTACATCCGTGCCTTCCAGTGGACGGGT CCCCGAAACGACATGTACTCTTGGGGCCAGTTCATGTCCGACTTCTTTGGTGTGAAGTT CGTGCGCGTCGGCAACGAGAAGCTGTACAAGCCAAAGAAGGGCGCGAACGTCATCTAC CTCTGCAACCACCGCTCCTGGGCCGACTTTTTCTGCGACGTCTACCTCAGGAGGCAA CGCCGCTCTCCTGTGCGCATGCTGGTGTTCATCTTTCCGTTTTTCATGTCCAGCGT CGTGATCCTGAAGGGCGTCGTGCTGTTCAAGCGGGGCAAGGTGGTCGACAAGGACGCG TTCAACAAGCGTCTCGACGGCAAGCTGGCGGACTCGCCCATCCCTGGCCTCCTGGTCTA CCCAGAGGGCACGCGGAGCCTGAAGGACCTGTCTCTGCCGCTGAAGCGCGGGATGCTC TACTTCGCGCATAGCCGCAACATTCCGGTGCAGATCGTGGTCAGCTCTGGAAAGGAGTA CGTCCTCACCAGAGAAGAAGATCCGCGCCCGCATCGGCCGCACCGTCGTGTCCACCTTCT CCGCGAAGATCACCCCGTCCGACTTCCCATCGTGCGAGGCCTTCCTGGAGCACGTGCA AAAGGAGTGGGACCGCCTGTGGCCCGCCGTCTTACGGCCGACGCTGACAAGGCCCC GTCCTGGTGAACGAGGACATCCCGATGCACGACTACGCGAACACCATGCGATGGGCGC AGCTCGGAATGGCCGTGCTCTTACCACGGTGGCGACGATCATGTTCTCTGAGCTGG AAGGTCGTGGACCTGATTTTCGGCATCTTCGGGCCCCTGAAGCAGGTCCTGTACATGCT CCTGTTACCTGGGTGTCGGTGTGCGCTCGTGGCGAGCTTCTGGGGCCAAAGCATCTG CCCAAGGGGGTCTGCCCTCCCCCGTCTGCCCTGCCCTGCCCTGGCGCGGGCCACGCCCC CGTCCCAGGAGCCGTCCAGCTCGGGGTCTAAGTGA <u>Ag</u>taccacgcccgcgcgcgcacgtgacctgtctctc gaggcgccctgttctgccttgcgaaacaagcccctggagcatgcgtgcatgatcgtctctgcgcgcccgccgcgcggttctgcctctgcg ggcgccccggccgcggggcgccattgaaattgttgcacccacccctgacagattgaggccagggcaggaagcgcttgatgagaggt acaggagtcgaagtaactgaaagtgtttatgataactaacaacaagggctgttctgcccagcgaatgacaagaacaagattccacattccgtg tagaggcttgccatcgaatgtgagcggcgggcccgccgacccgacaaaaccttacgacgtgtaagaaaaacgtggcgggcactgtccc tgtagcctgaagaccagcagagacgacggaagcatcacagcagccggcccgctctcgaacagagcgcggcgccgcacatgat acctgcaggacagcgccatgccacgccccttcatggttcaagtacgattacgggtgttgattgtgtgtttgttcgtagtctgcatgggt tagaataatacacttgatttcttctcagggcaatctcggtgttcgcaggttcaacccatttcggagtctcaggtcagcgcgcaat gaccagccgctacttcaaggacttgcacgacaacgcccaggtgagctatgtttaggacttgattggaaattgtcgtcgacgcatatc gegctccgcgacagcaccacaagcaaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgttgtgatcgtcgccgagatccg ccggtgtctcctgcgttacgagctgaccaagcaccctgacgtccgggtacgcgagctgagattcgattagacataaattgaagatta aacccgtagaaaaattgatggtcgcgaaactgtctcgattgcaagaaattgatcgtctcactccgaggtcgccatcatcgagc aggcggtgtctccggcgccggcgccctggtggggggacagctgttctcgccatgtgtgtacgtagaaggatgaatttcagctggtt ttcgttgacagctgtttgtgcatgattgtttcagactattgtgaatgttttagatttcttaggatgcatgattgtctgcatgcgact

[0352] The construct can be written as
5’Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:MyarcLPAAT2:PmPGH::3’Thia4.
Proceeding in the 5’ to 3’ direction, bold, lowercase sequences represent genomic DNA from
CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the C.

reinhardtii β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *MyarcLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. A PmG3PDH promoter from *P. moriformis*, indicated by the boxed italicized text, drives the expression of the *MyarcLPAAT2*. The initiator ATG and terminator TGA codons of the *MyarcLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0353] Table 103 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1227. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 103: Screen of primary transformants of CHK22 with pCHK1227.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1227-1	0.03	1.23	27.32	2.80	59.58	6.95	0.49	0.25	0.00	2.42
pCHK1227-2	0.03	1.24	27.17	2.82	59.77	6.87	0.49	0.25	0.00	2.48
pCHK1227-3	0.03	1.24	27.37	2.74	59.53	6.98	0.49	0.24	0.00	2.49
pCHK1227-4	0.03	1.26	27.46	2.88	59.40	6.87	0.49	0.25	0.00	2.05
pCHK1227-5	0.03	1.26	27.24	2.85	59.45	7.10	0.49	0.25	0.00	2.87
pCHK1227-6	0.04	1.30	27.22	2.86	59.09	7.34	0.52	0.25	0.00	2.34
pCHK1227-7	0.03	1.26	27.32	2.83	59.30	7.12	0.50	0.25	0.00	2.89
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 50: Expressing *Chlamydomonas* sp. UWO_241 (*CuwoLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0354] *CuwoLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1228 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 104**.

Table 104: Integrative sequences for the transformation of *P. moriformis* with pCHK1228 encoding *CuwoLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
80	ccctcaactgcgacgtgggaaccttctccgggcaggcgatgtgcgtgggttgccctctggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgggtgccacttcgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgcgacgtcgagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgctccagatcgacagatagcgaccatgtgtgctgcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgtacaattcctgtcaccatcgccatggagcaggctccacttagattcccgate accacgcacatctcgtaatagtcatcttcgtgtcttcgatcaatctcaagtgagtgatggatcttggttgacgatcgcggtatg ggtttgcgccgtggctgcagggtctgccaaggcaagtaaccagctctctccccgacaatactctcgaggcaaaagccggtca cttgccttcagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgctgctgctgaccgtt ccccagcctctggcgtccccgccccaccagccacgccgcggcgagtcgctgccaaggctgtctcggaCTTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCATTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACAATGctgtgcaggccttctgttctgtgctggccggcttcgccccaagatcagcg cctccatgacgaacgagacgtccgacggccccctgggtgacttcacccccaaagggctggaatgaacgacccccacggcctgtggta cgacgagaaggacgccaagtggcacctgtacttcagtagacaacccgaacgacacgtctgggggacgcccttgttctggggccacgcc acgtccgacgacctgaccaactgggaggacagccccatcgccatcgccccgaagcgcaacgactccggcgcccttccggctccatgg tggtggactacaacaacacctcggttcttcaacgacaccatcgacccgcccagcgctgctggccatctgacgtacaacaccccg gagtcggaggagcagtagatctcctacagcctggacggcgctacaccttcaccgagtagcagaagaacccccgtgctggccgcaact ccaccagttccgcgacccgaaggtcttctgtgtagacccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagtctggaagctggagtcggcttgcgaacgagggttctcggctaccagtagagtgccc cggcctgatcgaggtccccaccgagcaggacccccagcaagtcctactgggtgatgttcatctccatcaacccccggcggccggcggc ggctccttcaaccagtacttcgtcggcagcttcaacggcaccacttcgaggttcgacaaccagtcccgctgggtgacttcggcaag gactactacgccctgcagaccttcttaacaccgacccgacctacgggagcgccctgggcacgcgtgggctcccaactgggagtactc cgcttcgtgcccacaaacccctggcgctcctccatgtccctgtgctgcaagttctccctcaacaccgagtagcaggccaacccggagac ggagctgatcaacctgaaggccgagccgatectgaacatcagcaacgcccggccccctggagccggttcgccacaaacaccaggtgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcaccctggagttcgagctgggtgtagccgtcaacaccaccagac gatctccaagtccgtgttcgagacctctccctgtgttcaaggccctggagaccccgaggagtagctccgcatgggttcgaggtgtcc gcgtctccttcttctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttccaaacccgcatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtctactacaaggtgtacggcttctggaccagaacatctgagctgtacttcaacgacg gcgacgtcgtgtccaccaacacactattcatgaccaccgggaacgcccgtggctccgtgaacatgacgacgggggtggacaacctgttc tacatcgacaagtccaggtgcgcgaggtcaagtTAAAGACGCCCCGCGCGGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCTGGAGCATGCGTGATGATCG

SEQ ID NO:	Nucleotide Sequence
	<u>TCTCTGGCGCCCCGCCGCGGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC</u> <u>GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT</u> <u>TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTATGATAACTAACAACAAA</u> <u>GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAGATTCCACATTTCCTGTAGAGGC</u> <u>TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCCTTACGACGTGGT</u> <u>AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG</u> <u>GAAGCATCACAGCACAACCGGT</u><i>gcagcagcagctcggatagatcgacacactctggacgtggtcgtgtgatggac</i> <i>tgttgccgccacacttgctgccttgacctgtgaatatccctgccgtttatcaaacagcctcagtggttgcattgtgtgtacgcgctttg</i> <i>agtgctagctgctgtgctatttgcgaataccacccccagcacccttcctcgtttcatatcgcttgcateccaaccgcaactatctacgc</i> <i>tgctcgtatccctcagcgtgctcctgctcctgctcactgccctcgacagccttggttgggctccgctgtattctcctggtactgcaacc</i> <i>tgtaaacagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatca</i><u>TTGCGCCG</u> <u>CTCAGCCTACACGTCTTCTCCGATACCTTTCCCTCATTGCATTTTATGCCAGACTGGGTCCC</u> <u>AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC</u> <u>TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAAACCTGCCAGAAACAATTC</u> <u>AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTAGACGGGAAGCAACCCGC</u> <u>TTCCAGAAGCATCCGGACGGGGGTAGCGAGGCTGTGTCGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TGCACCCCGTGGGCCGGGCCCCCGGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTTACTCCTTAATCCTCGCTCCTCCTCATATACAACCACAAGTAATCAACTCACC</u> <u>A</u><u>ATGGCGGCCAACATGGCGACGTTCCCTTCTTTCTCCTGACCTTCTTTTCTGTACTG</u> <u>GTCCATGGCCATCGGCACCCCTGGTCGGACGGGTCAAGTTTTGGCAGTACAACGGCACC</u> <u>AACCGCAACGAGATGTTTCGTGTGGGCCAAGCTGTGCTCGGACTTTCTGGGCGTCAAGT</u> <u>ACCTGAAGACGGGCTCGGGCTCTCTCTACAAGAAGGAGCGCTGCGTGTACCTGAGCAA</u> <u>CCACCGCTCCTGGGCGGACTTCTTTGTGGACGTGTACCTGACCGAGGGCATCGCGCAG</u> <u>GCCATGGCGCGAACCATGGTCGGCATCGCCTTCCCCATGTTTATTGGCGCGGCTACCAT</u> <u>CCTGCGAGGCATCCTCCTGTTTAAGCGGGGCTCCATCGCCAACAAGGACAAGTTCAACA</u> <u>CCTGGCTCGATGACAAGCTGAACAGCTCCCCGTGCTCCTCTCTGCTCGTGTTCCTCGAG</u> <u>GGCCACCGAAGCACCAAGTCCACGAGCCTGCCGCTGAAGCGCGGCATGCTGTACTACG</u> <u>CGCATTCGCGCGGGCTGCCTGTGCAGGTGATCATGACGCGCGGGAAGGAGCTGGTGCT</u> <u>GTCGGAGAAGCACCATCACGCGGGCTACGGACCCAACTGATTACGGGCTTTTCCGAG</u> <u>CTGGTGCTGAGCAAGGATAAGGAGTTCGAGCAGTTTTGGACCGAGGTCCAGGCGATGT</u> <u>GGGACCGCATGTGGGCCGAGGTGTACAGCGTCGACCCCTCCACGGCGGTGCCTTACAC</u> <u>GCAGGACGTGCCCGGCAGCGGTGACCCCTCTCCATGTCGGTCCTGTACACCGTGTTTA</u> <u>CGGTGTGACCGTCATCTTTCTGCTCTCGACGCTCTGGATCATGGCGCGGATCTTCGGC</u> <u>TTTATGATCTCCCCGCTGCCCGCGATGCTCCAAATGGTGGTCTGGGGCGCCGTCCCGGT</u> <u>CACCTGGCGGTGTGTTCCGGGCCTCTATTATCGACAACGCCAAGACGTCTCGTCCG</u> <u>CCGTCATCAAGACCGCGAAGAAGGCGCAATGA</u><i>gtaccacgcccgcgcccgcacctgacctgttctctcagg</i>

SEQ ID NO:	Nucleotide Sequence
	<u>gcgctgttctgccttgcgaaacaagcccttgagcatcggtgcatgatcgtctctggcggcccgccgcgcggttctgcctcgcgggcg</u> <u>ccccggccgcgggggcgcattgaaattgttgcaaacccacctgacagattgagggcccaggcaggaaggcgttgagatggaggtag</u> <u>gagtcgaagtaactgaaagtgtttatgataactaacaacaagggtcgttctggccagcgaatgacaagaacaagattccacattccgtgtaga</u> <u>ggcttgccatcgaatgtgagcggcgggcgccgcggaccgcacaaaacccttacgacgtgtaagaaaaacgtgcgggcactgtccctgta</u> <u>gcctgaagaccagcaggagacgatcggaagcatcacagcagccggcccgccgtctcgaacagagcgcggcgccgcacgcatacct</u> <u>gcaggacagcgccatgccacgcccttgatggctcaagtacgattacgggtggtgattgtgtttgttcgtagtgtcatgtgtttag</u> <u>aataatacattgatttctgtcacggcaatctcggtgttcgcaggttcaacccatttcggagtctcaggtcagccgcgaatgac</u> <u>cagccgctactcaaggacttgcacgacaacgccagggtgagctatgttaggacttgattggaaattgtcgtcgacgatattcgcg</u> <u>ctccgcgacagcaccacaagcaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgttgatcgtcgccggcgatccgcg</u> <u>gtctgtcgtcgcttacgagctgaccaagcaccctgacgtccgggtacgcgagctgagattcgattagacataaattgaagattaaac</u> <u>ccgtagaaaaattgatggtcgcaaatgtgtcgtgattgcaagaaattgatcgtctccactccgaggtcgccatcatcgagcagg</u> <u>gcgtgtctccggcgccgcctggctggggggacagctgttctcgccatgtgtgtacgtagaaggatgaatttcagctggttttcg</u> <u>ttgcacagctgtttgtcatgatttttcagactattgtgaatgttttagatttcttaggatcatgatttctcatcgact</u>

[0355] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:CuwoLPAAT2:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *CuwoLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *CuwoLPAAT2*. The initiator ATG and terminator TGA codons of the *CuwoLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0356] **Table 105** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1228. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct

transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 105: Screen of primary transformants of CHK22 with pCHK1228.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1228-1	0.03	1.24	27.29	2.77	59.73	6.84	0.48	0.24	0.00	2.9
pCHK1228-2	0.03	1.24	27.12	2.79	59.89	6.87	0.47	0.24	0.00	3.33
pCHK1228-3	0.03	1.26	27.24	2.74	59.63	7.02	0.47	0.23	0.00	3.65
pCHK1228-4	0.03	1.25	26.79	2.87	60.30	6.75	0.46	0.24	0.00	3.45
pCHK1228-5	0.03	1.26	27.18	2.89	59.84	6.73	0.48	0.24	0.00	2.79
pCHK1228-6	0.04	1.26	27.24	2.78	59.84	6.80	0.47	0.22	0.00	3.39
pCHK1228-7	0.03	1.27	27.34	2.84	59.42	6.95	0.51	0.25	0.00	3.04
CHK22 Control	0.04	1.27	27.29	2.45	59.08	7.45	0.54	0.23	0.00	1.5

Example 51: Expressing *Chlamydomonas moewusii* (*CmoeLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0357] *CmoeLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct, pCHK1231, contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 106**.

Table 106: Integrative sequences for the transformation of *P. moriformis* with pCHK1231 encoding *CmoeLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
81	ccctcaactgcgacgctgggaaccttctccgggcaggcgatgtgcgtgggttgcctcctggcagcgctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcggttgccacttcgtccagagacggcaagtcgccatcctctgcgtgtgtggcgcgacgctgcagca gtccctctgcagcagatgagcgtgactttggccatttcacgcactcgagtgtacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgctgatttcgctccagatgcacagatagcgaccatgttgcgtcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattcctgtcacccatcgcatggagcagggtccacttagattcccgatc acccacgcacatctcgtaatagtcattcgttctgtcttcgatcaatctcaagtgcgtgcgtggtggtgacgatgcggtatg ggtttgcgcgctggctgcagggtctgccaaggcaagctaaccagctcctctcccgacaatactctcgaggcaaaagccggtca cttgcttccagattgccaataaactcaattatggcctctgtcatgccatccatgggtctgatgaatggtcacgctcgtgtcctgacggtt ccccagcctctggcgctccctgccccgcccaccagcccgccgcggcagtcgctgccaaggctgtctcggaCTTTCTTGC GCTATGACACTTCCAGCAAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTAAATAGCCAGGCCCCCGATTGCAAAGAC

SEQ ID NO:	Nucleotide Sequence
	ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACCTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCTCTTCGTTTCA GTCACAACCCGCAAACCTGTACA ATGctgtctgcaggccctctgttctctgtggccggcttcgcccaagatcagcg cttccatgaagaaacgagacgtccgaccgccctgtgtgacttcaccccccaacagggtgatgaacgacccaacggcctgttgta cgacgagaaggagcgaagtggcactgtacttcagtacaacccgaacgacaccgtctgggggacgccctgttctggggccacgc acgtccgacgacctgaccaactgggaggaccagcccatcgccatcgcccgaaagcgcaacgactccggcgcttctccggctccatgg tggtggactacaacaacacctecggcttctcaacgacaccatcgaccgcgcagcgcgtgcgtggccatctggacctacaacccccg gagtcggaggagcagtaactctctacagcctggacggcgctacaccttcaccgagtaaccagaagaacccccgtgtggccccaact ccaccagttccgcgacccgaaggcttctgtgtacgagccctccagaagtggatcatgaccgcggccaagtccaggactacaagat cgagatctactctccgacgacctgaagtctggaagtgtgagtcgcgttcgccaacgaggcgctctcggtaccaggtacgagtgccc cggcctgatcagggtccccaccgagcaggacccagcaagtctactgtggtgatgttcacatcaacccccggcgccccggcggc ggctccttcaaccagtacttctgtcgagcttcaacggcaccacttcgagcgcttcgacaaccagtcgccgtgtgtgacttcgcaag gactactacgcccgtcagacaccttctcaacaccgacccgacctacgggagcgccctgggcatcgctgggectccaactgggagtactc cgcttcgtgcccaccaacccctggcgctctctcatgtccctcgtgcgcaagtctccctcaacaccgagttaccaggccaacccggagac ggagctgatcaaacctgaaggccgagccgactctgaacatcagcaacgccggccccgtgagccggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgactgtccaacagcaccggcaccttgaggttcgagctggtgtacgccgtcaacaccaccagac gatctccaagtccgtgttcgaggacctctccctctgttcaaggccctggaggaccccgaggagtacctcccgatgggcttcgaggtgtcc gcgtcctcttctcttgaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaaccaaccgatgagcgtgaacaa ccagccctcaagagcgagaacgacctgtctactacaaggtgtacgcttctgtggaccagaacatctctggagctgtacttcaacgacg gcgacgtcgtgtccaccaaacacttactcatgaccaccgggaacgccctgggctcctgaacatgacgacgggggtggacaacctgttc tatatcgacaagtctcaggtgcgcgagggtcaagtgaactTAAGACGCCCCGCGCGGCACCTGACCTGTTCT CTCGAGGGCGCCTGTCTTGCCCTTGCGAAACAAGCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCCGCGCGGTTTGTCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA GGGTCGTTTCTTGCCAGCGAATGACAAGAACAAGATTCCACATTTCCGTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAAACCGGTcgacgacgactcgatagatcgcacactctggacgctggctgtgtgatggac tgttgcggcaactgtgtccttgacctgtgaatateccctgccgttttatacaaacgacctcagtggtttgatctgtgtgtacgcgcttttgcg agttgctagctgtgtgtctattttggaataacacccccagcatcccttccctcgtttatatacgtttcatcccaaccgcaacttatctacgc tgtctctctatccctcagcgctgtctctgtctctgtcactgcccctcgcacagccttggttgggtctcgctgtatttctctgtgtactgcaacc tgtaaaccagcactgcaatgtgtatgcacgggaagtgtgggatgggaacacaaatggacctaggaaataacaatcaITCGCCG CTCAGCCTACACGTCTTCTCCGATACCTTTCCCTCATTTGCATTTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGAATTGCTCGTGTGCGAGGCGGGGCACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCCTGACGACTGTAACCCCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC

SEQ ID NO:	Nucleotide Sequence
	<u>TTCCAGAAAGCATCCGGACGGGGGTAGCGAGGCTGTGTCTGAGCGCCGTGGGGATCTGGCC</u> <u>GATGAGGTGCCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA</u> <u>TCGACCCCCGTGGGCCGGGCCCCCGGACCCTATAACTAAAAGCCGACGCCAGCGCAAAACC</u> <u>ACAAACATTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCAC</u> <u>A</u>ATGGCCAGCTTCGTCGATCTCTGCTCCAGTTCCTTACTTTGGCCTGACGATCTTCTT TTTCTACTGGTCCCCACCCCTGGCGTCCATCGTCTACCGAATCCGCTTCGCGTCGGCGG GACCCCGCAAGGACATCTACGAGTGGACGACCGCCATGGTCCAGTTCCTCGGCGTGCG CATCCGTCACCAAGGCAAGCAGACCCTGTACAAGAAGGAGCGCTGCGTCTACCTGTGC AACCACCGCTCCTGGGCGGACTTTTTCGTGGACCTGCACCTGACCCAAGGCCTGGCGG CCCCATGAGCCGCACCATGGTGTGTTTTTCGCCTTCCCTGTGTTTCATGAGCATTGTCATC GTGCTCAAGGGCGTCTCTCTTCAAGCGCGGCACGATCGCCGACAAGACGAAGTTCA ACAAGTGGCTGGATGACAGCCTGGGCAACAGCCCGGTGTCTGCCTGCTCGTGTACCC CGAGGGCCACCGCTCTACGAAGGCGAAGTCGCTGCCGCTGAAGCGCGGTATGCTGTAC TACGCGCACAGCCGCGGCTGCCCGTGCAGATTATCCATACCGCGGGCAAGGAGGCCG TCCTGTCCGAGAAGCGCATGCGCGGAGCTACGGCCAGACCCTCGTCACGGGCTTCTC GCAGCCCATCCAGTCCAAGGACTTCAAGGACTTCGAGGCCTTCTCTGCGGAGGTCCAG CGGAGCTGGGACGCGCTCTGGACCGAGGTGTAATCCGCGAACCCTCCGCGCTGCCCC CGCTGGTGCAGGAGGACGTCCCCACCGCGGACTACCCGCCTTCCATGCGCACGGGCAT GCTCCTGGTCAGCCTGGCGGCCATCGCCCTGTTCTGGCCTTTGTGCGCTCCTGGCCT ACATCACGGGGCCCGGTGATGAGCCTGTTCCCCGCTCCCTCCGCGTCCCACTCATTCTC TTTAAGCTGTCTGGCACGCGGCCAGCTTCCTGTACTGCGGGGCCAAGGACACCCCTG GCAAGGTGAAGGCGGCGTGAgtaccacgcccggcgccgcacacgtgacctgttctctcagggcgccgtgttctgcctgcg aaacaagcccctggagcatgcgtgcatgatcgtctctggcgccccggcgcggtttgtcgcctcgcggcgccccggcgcgggggc gcattgaaattgttgcacacccacacgtgacagattgagggcccaggcaggaaggcggttgagatggaggtacaggagtcagtaactgaaag ttttatgataactaacaacaaagggtcgtttctggccagcgaatgacaagaacaagattccacatttccgtgtagaggcttgcacgaatgtga gcggcgggggcgccggacccgacaaaacccttacgacgtggttaagaaaaacgtggcgggcactgtccctgtagcctgaagaccagcagga gacgatcggaagcatcacgacagggcgccccgcgtctcgaacagagcgcgggcgccgcatcgatacctgcaggacagcgccatgcc acgccccttgatggcttcaagtacgattacgggttggtgattgtgtttgtgtagtgcagtggttagaataatacaacttgatttctt gtcacggcaatctcggttgcgcaggttcaacccatttcggagttcaggtcagccgcgaatgaccagccgtacttcaagga cttgacgacaacgccgaggtgagctatgttaggacttgattgaaattgtcgtgacgcatattcgctccgcgacagcaccacaa gcaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgtgtgatcgctggcgccgagtcgcccgtgtctgcgttcacga gctgaccaagcacctgacgtccgggtacgagctgagattcgattagacataaattgaagattaaacccgtagaaaaattgatg gtcgcgaaactgtgctgattgcaagaaattgatcgctcctcactccgaggtcgccatcagcagggcggttgcctccggcgggcg gcgctggctggggggacagctgttctcgccatgtgtgtacgtagaaggatgaatttcagctggttttcgtgacagctgtttgtgca tgatttgttcagactattgtgaatgttttagatttcttaggatgcagatttgcgtcatgcgact

[0358] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:CmoeLPAAT2:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *CmoeLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *CmoeLPAAT2*. The initiator ATG and terminator TGA codons of the *CmoeLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0359] **Table 107** shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1231. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 107: Screen of primary transformants of CHK22 with pCHK1231.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1231-1	0.03	1.19	26.09	3.30	60.84	6.59	0.52	0.31	0.00	1.55
pCHK1231-2	0.04	1.29	21.15	4.13	61.84	9.50	0.58	0.41	0.00	1.52
pCHK1231-3	0.03	1.34	26.69	2.91	58.84	8.26	0.50	0.24	0.00	4.45
pCHK1231-4	0.03	1.33	26.34	3.43	59.14	7.80	0.52	0.28	0.00	4.61
pCHK1231-5	0.03	1.24	26.27	3.30	60.38	6.81	0.55	0.28	0.00	1.53
pCHK1231-6	0.03	1.36	26.40	2.20	60.87	7.26	0.31	0.16	0.00	4.49
pCHK1231-7	0.03	1.27	26.59	3.12	59.06	7.91	0.53	0.27	0.00	4.61
pCHK1231-8	0.03	1.27	26.39	2.97	59.77	7.68	0.49	0.21	0.00	4.79
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

Example 52: Expressing *Oophila amblystomatis* (*OambLPAAT2*) in *P. moriformis* strain CHK22 using the PmG3PDH promoter.

[0360] *OambLPAAT2* was introduced into a *P. moriformis* strain CHK22. The expression construct pCHK1232 contained 5' and 3' homology arms to permit its targeted integration into the genome and is shown in **Table 108**.

Table 108: Integrative sequences for the transformation of *P. moriformis* with pCHK1232 encoding *OambLPAAT2*.

SEQ ID NO:	Nucleotide Sequence
82	ccctcaactgcgacgtgggaacctctccgggcaggcgatgtgcgtgggtttgcctccttggcacggctctacaccgtcgagtacgcc atgaggcggtgatggctgtgtcgttgcactctgtccagagacggcaagtcgtccatcctctgcgtgtgtggcgacgtcgagca gtccctctgcagcagatgagcgtgactttggcaatttcacgactcagtgtagacacaatccattttctaaagcaaatgactgctgatt gaccagatactgtaacgtgatttcgtccagatgcacagatagcgaccatgtgtgctgcgtctgaaaatctggattccgaattcgacc ctggcgctccatccatgcaacagatggcgacacttgttacaattcctgtcaccatcgccatggagcagggtccacttagattcccgatc accacgcacatctcgctaatagtcattctgtctgtctcgtatcaatctcaagtgtgtgcatggatcttgggtgacgatcgcggtatg ggtttgcgccgtggctgcagggtctgccaaggcaagtaaccagctcctctccccgacaatactctcgcaggcaaaagccggtca cttgccttccagatgccaataaactcaattatggcctctgtcatgcatccatgggtctgatgaatgggtcacgctcgtgtcctgaccgtt ccccagcctctggcgtccccctgccccgccaccagccccacgcgcggcagtcgctgccaaggtctgtcgggaCTTTCTTGC GCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTGC ATGCAACACCGATGATGCTTCGACCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCT CCGATGCCGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGAC ATTATAGCGAGCTACCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACA GGCCACTCGAGCTTGTGATCGCACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTTTCA GTCACAACCCGCAAACTGTACAATGctgctgcaggccttctgttctgctggtgcccggcttcgccccaagatcagcg cctccatgacgaacgagacgtccgacggccccctggtgeacttcacccccaaaggctggatgaacgacccccaaaggcctgtgga cgacgagaaggacgccaagtggcactgtactccagtacaacccgaacgacaccgtctgggggacgcccttgttctggggccacgcc acgtccgacgacctgaccaactgggaggaccagccccatcgccatcgccccgaagcgcaacgactccggcgcccttctccggctccatgg tggtggaactacaacaacacctccggcttcttaacgacacccatcgacccgcgcagcgctgcgtggccatctggacctacaacaccccg gagtcgaggagcagtagatctctacagcctggacggcggtacaccttcaccgagtagcagaagaaccccgctgctggccgcaact ccaccagttccgcgacccgaaggtcttctgtacgagccctccagaagtggatcatgaccgggccaagtccaggactacaagat cgagatctactcctccgacgacctgaagtcctggaagctggagtcgcgttcgccaacgagggttctcggctaccagtagagtgccc cggcctgacgaggtccccaccgagcaggacccccagcaagtcctactgggtgatgttcattccatcaaccccgcgccccggccggc ggctccttcaaccagtactctgcggcagcttcaacggcaccacactcgaggccttcgacaaccagtcccgctgggtgacttcggcaag gactactacgccctgacgaccttcttaacaccgacccgacctacgggagcgccctgggcatcgctgggctcccaactgggagtactc cgcttcgtgccccaccaacccctggcgctcctccatgtccctgtgcgaagtctccctaacaccgagtagcaggccaacccggagac ggagctgatcaacctgaagccgagccgacccgagcaacgcccggccctggagccggttcgccaccaacaccacgttgac gaaggccaacagctacaacgtcgacctgtccaacagcaccggcacccctggagttcgagctggtgtacgccgtcaacaccaccagac

SEQ ID NO:	Nucleotide Sequence
	gatctccaagtcctgttcgaggacctctccctctggttcaaggcctggaggaccccaggagtagctcccatggcttcgaggtgtcc gcgctctcttcttctggaccgcgggaacagcaaggtgaagttcgtgaaggagaacccctacttcaccaaccgcatgagcgtgaacaa ccagcccttcaagagcgagaacgacctgtcctactacaaggtgtacggcttctggaccagaacatectggagctgtacttcaacgacg gcgacgtcgtgtccaccaacacctattcatgaccaccgggaacgccctgggtccgtgaacatgacgacgggggtggacaacctgttc tatacgcacaagttccaggtgcgcgaggtcaagtgaactTAAGACGCCCCGCGCGGCGCACCTGACCTGTTCT CTCGAGGGCGCCTGTTCTGCCTTGCGAAACAAGCCCCCTGGAGCATGCGTGCATGATCG TCTCTGGCGCCCCGCGCGGGTTTGTGCGCCCTCGCGGGCGCCGCGGCCGCGGGGGC GCATTGAAATTGTTGCAAACCCACCTGACAGATTGAGGGCCAGGCAGGAAGGCGT TGAGATGGAGGTACAGGAGTCAAGTAACTGAAAGTTTTTATGATAACTAACAACAAA GGGTCGTTTCTGGCCAGCGAATGACAAGAACAAAGATTCCACATTTCCTGTAGAGGC TTGCCATCGAATGTGAGCGGGCGGGCCGCGGACCCGACAAAACCTTACGACGTGGT AAGAAAAACGTGGCGGGCACTGTCCCTGTAGCCTGAAGACCAGCAGGAGACGATCG GAAGCATCACAGCACAACCGGTgcagcagcagctcggatagatcgcacacactctggacgtggtgctgtgatggac tgttgcggccacactgtcgtcttgacctgtgaatatccctgccgttttatcaaacagcctcagtggtgttgatcttgtgtacgcgcttttgcg agttgctagctgtgtgtctatttgcgaatacaccceccagcacccttccctcgtttcatatcgttgcacccaaccgcaacttatctacgc tgtctgtctatccctcagcgtgtcctcgtcctcgtcactgccctcgacagccttggttgggtccgctgtattctcctggtactgcaacc tgtaaaccagcactgcaatgctgatgcacgggaagtagtgggatgggaacacaaatggacctaggaaataacaatcaTTCGCGG CTCAGCCTACACGTCTTCTCCGATACCTTTCCTCATTGCATTTATGCCAGACTGGGTCCC AGCCTGGGTGGGTGCTCCCGCTCGATTGCTCGTGTGCGAGGCGGGGACCCCCGCTCTC TCTATTTATCACTGCCTCTCCCCGACCAACCCTGACGACTGTAAACCTGCCAGAAACAATTC AGCCTCAACAAGCCGAGTTGTGCACAAGGGAGACTAATTTTTTAGACGGGAAGCAACCCGC TTCCAGAAAGCATCCGGACGGGGGTAGCGAGGCTGTGTGCGAGCGCCGTGGGGATCTGGCC GATGAGGTGCCCGAAACCCGTGTACAGCTCAGCGGCTGGGATCATCGACCCCCGGGATCA TCGACCCCCGTGGGCCGGGCCCCCGACCCCTATAACTAAAAGCCGACGCCAGCGCAAAACC ACAAACATTTACTCCTTAATCCTCGCTCCTCCTTCATATACAACCACAAGTAATCAACTCACC AATGCCCCCTGGCGCGCTCCAGCTCCTCGTCCGCGCTCCTGCTCTGGCTGCTCCGCCTGC CGTACTTTCTGCTCACCATCACCTTTTTCTACTGGTCCGCCACGATCGGCGCCCTGATCT ACCGCGTCCGCTTCCTGTCTACCGGGCCGCGCAACGACATGTACGACTGGACCCCTGCTC ATGACCTCGTTCTGGGTCTCCACTTCCACCAACTGGGTGCCCAACGCCTGCACTGCGC GCCCCGAGTCGTGTTTCTGGCCAACCACCGAAGCTGGGCGGACTTTTTCTGTCGACATCC ACCTGACCCAGGGGACCGCCGCTCCCATGTCCCGCGCGCTGGTGGCTTTTGCGTTTCCT GTGTTATGTGCACCGCCTGGCTCCTGCGCGGACTGATTATCTTCAAGCGCGGCGCGAT CGAGGACAAAAAGAAGTTCAACTCGTGGCTGGACGAGCAGCTCGACGCGTCCGCGGGC TCGAACCTCCTGGTGTACCCCGAGGGACACCGCAGCACCCGACCCACCTCCCTCCCACT GAAGCGCGGCATGCTGTACTACGCCCACTCCCGCGGCCTGCCCGTGCAAATTATCATGA CCGCTGGCAAGGAGTCTGTCTGTCGGAGAAGCACCAGTGCGCGTCTTTGGCCAACA CCTCCTGACCAGCTTCAGCCCCGTGATCTCGTCTAAGGACAAGGACTTCAGAGGTGTTCCG

SEQ ID NO:	Nucleotide Sequence
	<i>CTACGGAGGTCCAGCAACGCTGGGATTGCTGTGGCGCGAGGTGTTAGCACGTCGAC</i> <i>CCAAGGCGTCCCCCTCCTGCACCAGGCCAGCGTCCCCTCTTCGACTACCTCCCCTG</i> <i>TCCGCACGCACATGGCTCTCGCTTCCAGCGCCTTCATCTGATGCTGCTCCTGGCCGCG</i> <i>GGCGCGGCCGTCCTGGCCGTCCGCGCTACCCTGGCCCTGATGCCGCCCGTCTCCAGG</i> <i>TCGTGGTCCTCGCTGCCAGCTCCCTCCTGATGCTCGCGTCCTTTCTGACGTCGGCTGCC</i> <i>ATCACCTCGTCCACCACGACCACGACCACGACCACGCGACAGCCCCAGGCTGTGGCCG</i> <i>ACAAGGGCTCGAAGGAGTCTTAG</i><u><i>gtaccacgccgcgcgcgcacctgacctgttctctcaggcgccctgttctgct</i></u> <u><i>tgcgaacaagccctggagcatgcgtgcatgctgtctctggcgccccgccgcgcggtttgtgcctcgcggcgccccggcgccggg</i></u> <u><i>ggcgcatgaaattgttgcacaacccacctgacagattgaggcccgagcaggaaggcggttgagatggaggtacaggagtcaagtaactga</i></u> <u><i>aagttttatgataactaacaacaagggtcgtttctggccagcgaatgacaagaacaagattccacatttccgtgtagaggcttccatcgaat</i></u> <u><i>gtgagcgccggcgccgcggaccgacaaaaccttacgacgtgtaagaaaaacgtggcgccgactgtccctgtagcctgaagaccagc</i></u> <u><i>aggagacgatcggaagcatcacagcacaggccggccccgcgtctcgaacagagcgcgccggccgcatcgatactgcaggacagcgcc</i></u> <u><i>atggcacgccctttgatggctcaagtacgattacgggtgttggtgtgtgtgtgtgtagtgcagtggttagaataatacacttgat</i></u> <u><i>ttcttctcacggcaatctcggttgcgcagggtcaacccatttcggagtctcaggtcagccgcgcaatgaccagccgctacttcaa</i></u> <u><i>ggacttgcacgacaacgccgaggtgagctatgtttaggacttgattggaattgtcgtcgacgcataattcgctccgcgacagcacc</i></u> <u><i>caagcaaaatgtcaagtgcgttcgatttgcgtccgaggtcgatgttgcgtgcgcggatccgcggtctgtcctgcgtta</i></u> <u><i>cgagctgaccaagcacctgacgtccgggtacgcgagctgagattcattagacataaaattgaagattaaacccgtagaaaaatttg</i></u> <u><i>atgttcgcgaaactgtcgtcattgcaagaaattgatcgtctccactccgaggtgccatcatcgagcaggcggttgcctccggcg</i></u> <u><i>gcggcgctggctgggggacagctgttctcgccatgtgtgtacgtagaaggatgaatttcagctggtttcgttgcacagctgtttgt</i></u> <u><i>gcattgttttcagactattgtgaatgttttagattcttaggatgcattgtgtcgtgcgact</i></u>

[0361] The construct can be written as

5'Thia4::CrTUB2:ScSUC2:PmPGH:CvNR:PmG3PDH:*OambLPAAT2*:PmPGH::3'Thia4.

Proceeding in the 5' to 3' direction, bold, lowercase sequences represent genomic DNA from CHK22 that permit targeted integration at the Thia4 locus via homologous recombination, the *C. reinhardtii* β -tubulin promoter driving expression of the yeast sucrose invertase gene (conferring the ability of CHK22 to metabolize sucrose) is indicated by boxed uppercase text. The initiator ATG and terminator TAA for invertase are indicated in uppercase, bold italics while the coding region is indicated in lowercase italics. The *P. moriformis* PmPGH 3'-UTR that enables amplification of the *OambLPAAT2* gene, is indicated by uppercase underlined text followed by a linker, indicated by lowercase, bold italics, which contains a *C. vulgaris* nitrate reductase 3'-UTR. The *P. moriformis* PmG3PDH promoter, indicated by the boxed italicized text, drives the expression of the *OambLPAAT2*. The initiator ATG and terminator TAG codons of the *OambLPAAT2* are indicated by uppercase, bold italics, while the remainder of the gene is

indicated by bold italics. The PmPGH 3'-UTR is indicated by lowercase underlined text followed by the CHK22 Thia4 genomic region indicated by bold, lowercase text.

[0362] Table 109 shows the fatty acid/TAG profiles of seven primary transformants of CHK22 transformed with pCHK1232. The *P. moriformis* base strain CHK22 is shown as a non-transgenic control. Strains were grown for 120 hrs in a 50-mL conical tube at 200 rpm at 28 °C in a lipid production medium. Biomass was harvested, lyophilized to dryness, and subjected to direct transesterification to generate FAMES for subsequent quantitation and characterization by GC/FID. TAG analysis was conducted using the LCMS method as described above.

Table 109: Screen of primary transformants of CHK22 with pCHK1232.

Sample	FAME Profiles									TAG Profile m/z 577/603 (OP:OO)
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3 alpha	C20:0	C20:1	
pCHK1232-1	0.15	3.52	26.66	2.19	48.87	15.20	1.38	0.28	0.00	2.45
pCHK1232-2	0.08	2.61	27.32	2.45	58.12	7.55	0.34	0.21	0.00	1.86
pCHK1232-3	0.07	2.31	27.85	4.06	57.08	6.64	0.59	0.29	0.00	1.54
pCHK1232-4	0.04	1.37	25.80	3.54	60.00	7.29	0.55	0.29	0.00	2.31
pCHK1232-5	0.05	1.67	27.41	2.92	58.02	8.15	0.40	0.23	0.00	1.99
pCHK1232-6	0.03	1.27	26.15	3.51	60.39	6.87	0.52	0.25	0.00	2.74
pCHK1232-7	0.04	1.32	27.26	2.73	55.53	10.51	0.76	0.37	0.00	1.54
pCHK1232-8	0.07	1.97	25.97	3.09	55.03	11.16	0.91	0.38	0.00	2.09
CHK22 Control	0.04	1.4	25.8	2.69	61.83	6.51	0.36	0.16	0	1.5

[0363] The amino acid sequences of various LPAATs disclosed herein were compared with the amino acid sequence of CrLPAAT2 to generate a sequence percent identity (%). The results are shown in Table 110. The resulting phenotypes of expressing these LPAATs in the non-transgenic cell line CHK22 are indicated with the OP:OO ratio.

[0364] In some embodiments, a microalgal cell provided herein comprises an exogenous gene that encodes for an LPAAT in Table 110. In some embodiments, the exogenous gene can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a LPAAT in Table 110.

Table 110: Percent identity against CrLPAAT2 and OP:OO Ratio

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
CrLPAAT2	<i>Chlamydomonas reinhardtii</i>	XP_001699586.2	325 aa	100.0	3.45
CiLPAAT2	<i>Chlamydomonas incerta</i>	KAG2426747.1	325 aa	89.8	2.21
VrLPAAT2	<i>Volvox reticuliferus</i>	GIM16764.1	156 aa	80.8	1.69
ChsLPAAT2	<i>Chlamydomonas schloesseri</i>	KAG2454274.1	378 aa	77.2	1.99
EdLPAAT2	<i>Edaphochlamys debaryana_SRR13719274</i>	SRR13719274	324 aa	76.9	1.8
VitrLPAAT2	<i>Vitreochlamys sp. CL-2021_SRR13719283</i>	SRR13719283	328 aa	70.2	4.3
CchaLPAAT2	<i>Colemanosphaera charkowiensis_SRR13719279</i>	SRR13719279	324 aa	69.2	2.6
VaLPAAT2	<i>Volvox africanus</i>	GIL63906.1	324 aa	69.2	1.96
AgLPAAT2	<i>Astrephomene gubernaculifera</i>	GFR47784.1	354 aa	69.1	2.21
GvirLPAAT2	<i>Gonium viridistellatum_SRR13719236</i>	SRR13719236	326 aa	68.8	3.6
VaulLPAAT2	<i>Vitreochlamys aulata_SRR13719273</i>	SRR13719273	325 aa	68.7	4.6
GmulLPAAT2	<i>Gonium multicoccum_SRR13719234</i>	SRR13719234	324 aa	68.6	4.2
VcomLPAAT2	<i>Volvolina compacta_SRR888100</i>	SRR888100	326 aa	68.5	4.4
PjapLPAAT2	<i>Pleodorina japonica_SRR13719268</i>	SRR1371926	324 aa	67.7	2
PmorLPAAT2	<i>Pandorina morum_SRR13719246</i>	SRR13719246	325 aa	67.2	2
VbolLPAAT2	<i>Volvolina boldii_SRR13719245</i>	SRR13719245	325 aa	66.9	2.1
EcylLPAAT2	<i>Eudorina cylindrica_SRR13719256</i>	SRR13719256	325 aa	66.3	2
VcarfLPAAT2	<i>Volvox carteri f. weismannia_SRR13719248</i>	SRR13719248	326 aa	65.4	3.4

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
VferLPAAT2	<i>Volvox ferrisii_SRR13719278</i>	SRR13719278	325 aa	65.4	3.8
GoctLPAAT2	<i>Gonium octonarium_SRR13719233</i>	SRR13719233	351 aa	65.2	1.63
VsteLPAAT2	<i>Volvulina steinii_SRR13719272</i>	SRR13719272	326 aa	63.3	1.92
EdLPAAT2	<i>Edaphochlamys debaryana</i>	KAG2489453.1	325 aa	60.1	3.78
CeLPAAT2	<i>Chlamydomonas eustigma</i>	GAX78532.1	253 aa	55.3	1.59
Ch_CCAC2762_LPAAT2	<i>Chlamydomonas sp. CCAC2762_B_ERR3487411</i>	ERR3487411	386 aa	48.6	4.59
VcLPAAT2	<i>Volvox carteri_98847</i>	XP_002957774.1	303 aa	47.5	1.58
CnivLPAAT2	<i>Chlamydomonas nivalis_SRR12517273</i>	SRR12517273	360 aa	45.8	1.59
CmoeLPAAT2	<i>Chlamydomonas moewusii_SRR13719241</i>	SRR13719241	336 aa	45.4	4.79
PedLPAAT2	<i>Pedinophyceae sp.YPF-701</i>	CAG9465508.1	340 aa	45.4	4.47
MyarcLPAAT2	<i>Microglena sp.YARC_SRR18294403</i>	SRR18294403	362 aa	45.1	2.87
DsalLPAAT2	<i>Dunaliella salina_SRR9129444</i>	SRR9129444	335 aa	44.5	2.06
DsLPAAT2	<i>Dunaliella salina</i>	KAF5838861.1	333 aa	44.2	2.35
VcLPAAT2	<i>Volvox carteri f. nagariensis_120491</i>	XP_002947580.1	624 aa	43.4	1.59
OambLPAAT2	<i>Oophila amblystomatis_SRR5445907</i>	SRR5445907	359 aa	42.4	2.74
ChdeLPAAT2	<i>Chlorella desiccata</i>	KAH7617329.1	372 aa	41.5	1.71
SceLPAAT2	<i>Scenedesmus sp. NREL 46B-D3</i>	KAF6259536.1	358 aa	41.4	1.64
ChsoLPAAT2	<i>Chlorella sorokiniana</i>	PRW57325.1	363 aa	41.3	1.53

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
CuwoLPAAT2	<i>Chlamydomonas sp.UWO_241_SRR13696614</i>	SRR13696614	341 aa	40.6	3.65
McLPAAT2	<i>Micractinium conductrix</i>	PSC72189.1	426 aa	40.3	1.55
RsLPAAT2	<i>Raphidocelis subcapitata</i>	GBF93205.1	380 aa	39.8	1.58
ChvaLPAAT2	<i>Chlorella variabilis</i>	XP_005852243.1	357 aa	39.8	1.58
ChvuLPAAT2	<i>Chlorella vulgaris</i>	KAI3438340.1	360 aa	39.5	1.63
ApLPAAT2	<i>Auxenochlorella protothecoides</i>	XP_011401335.1	385 aa	37.0	1.58
ChprLPAAT2	<i>Chloropicon primus</i>	QDZ21586.1	335 aa	32.9	1.62
CosLPAAT2	<i>Coccomyxa subellipsoidea</i>	XP_005647638.1	197 aa	30.2	1.63
BpraLPAAT2	<i>Bathycoccus prasinos_ERR2041002</i>	ERR2041002	334 aa	29.1	1.66
OILPAAT2	<i>Ostreococcus lucimarinus</i>	XP_001421357.1	286 aa	26.8	1.63
PproLPAAT2	<i>Pycnococcus provasolii_ERR2041000</i>	ERR2041000	326aa	22.7	1.64
NoLPAT4	<i>Nannochloropsis oceanica</i>	BAW99690.1	445 aa	15.3	1.55
CrLPAAT1	<i>Chlamydomonas reinhardtii</i>	XP_042921325.1	332 aa	13.6	1.59
CrLPAAT1- Δ CST*	<i>Chlamydomonas reinhardtii</i>	XP_042921325.1	302aa	13.6	1.69
Sll1848	<i>Synechocystis sp.PCC6803</i>	WP_010873224.1	225 aa	13.3	1.6
EcplsC	<i>Escherichia coli</i>	HBN6599230.1	245 aa	12.4	1.59
AGPAT1	<i>Homo sapiens</i>	NP_001358366.1	283 aa	12.1	3.6
BnBAT2- Δ CST*	<i>Brassica napus</i>	NP_001302928.1	258 aa	11.9	1.9

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
NoLPAT3	<i>Nannochloropsis oceanica</i>	BAW99691.1	482 aa	11.1	1.6
BnBAT2	<i>Brassica napus</i>	NP_001302928.1	344 aa	10.3	1.57

[0365] The amino acid sequences were compared with the amino acid sequence of PedLPAAT2 to generate a sequence percent identity (%). The results are shown in **Table 111**. The resulting phenotype of expressing these LPAATs in CHK22 is indicated with the OP:OO ratio.

[0366] In some embodiments, a microalgal cell provided herein comprises an exogenous gene that encodes for an LPAAT in **Table 111**. In some embodiments, the exogenous gene can comprise a sequence with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a LPAAT in **Table 111**.

Table 111: Percent identity against PedLPAAT2 and OP:OO Ratio

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
PedLPAAT2	<i>Pedinophyceae sp. YPF-701</i>	CAG9465508.1	340 aa	100	4.47
VcomLPAAT2	<i>Volvulina compacta_SRR888100</i>	SRR888100	326 aa	45.9	4.4
CmoeLPAAT2	<i>Chlamydomonas moewusii_SRR13719241</i>	SRR13719241	336 aa	45.9	4.79
CchaLPAAT2	<i>Colemanosphaera charkowiensis_SRR13719279</i>	SRR13719279	324 aa	43.8	2.6
VaLPAAT2	<i>Volvox africanus</i>	GIL63906.1	324 aa	43.5	1.96
VferLPAAT2	<i>Volvox farrisii_SRR13719278</i>	SRR13719278	325 aa	43.5	3.8
GvirLPAAT2	<i>Gonium viridistellatum_SRR13719236</i>	SRR13719236	326 aa	43.5	3.6
VcarfLPAAT2	<i>Volvox carteri f. weismannia_SRR13719248</i>	SRR13719248	326 aa	43.5	3.4
VsteLPAAT2	<i>Volvulina steinii_SRR13719272</i>	SRR13719272	326 aa	43.5	1.92

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
VcLPAAT2 V2	<i>Volvox carteri f. nagariensis_120491</i>	XP_002947580.1	624 aa	43.5	1.59
CrLPAAT2	<i>Chlamydomonas reinhardtii</i>	XP_001699586.2	325 aa	43.2	3.45
VbolLPAAT2	<i>Volvulina boldii_SRR13719245</i>	SRR13719245	325 aa	43.2	2.1
AgLPAAT2	<i>Astrephomene gubernaculifera</i>	GFR47784.1	354 aa	42.9	2.21
CiLPAAT2	<i>Chlamydomonas incerta</i>	KAG2426747.1	325 aa	42.9	2.21
VitrLPAAT2	<i>Vitreochlamys sp. CL-2021_SRR13719283</i>	SRR13719283	328 aa	42.9	4.3
Ch_CCAC2762_LPAAT2	<i>Chlamydomonas sp. CCAC2762_B_ERR3487411</i>	ERR3487411	386 aa	42.9	4.59
EdLPAAT2	<i>Edaphochlamys debaryana</i>	KAG2489453.1	325 aa	42.6	3.78
ChsLPAAT2	<i>Chlamydomonas schloesseri</i>	KAG2454274.1	378 aa	42.6	1.99
EcyLPAAT2	<i>Eudorina cylindrica_SRR13719256</i>	SRR13719256	325 aa	42.4	2
GoctLPAAT2	<i>Gonium octonarium_SRR13719233</i>	SRR13719233	351 aa	42.4	1.63
PjapLPAAT2	<i>Pleodorina japonica_SRR13719268</i>	SRR1371926	324 aa	42.4	2
GmulLPAAT2	<i>Gonium multicoccum_SRR13719234</i>	SRR13719234	324 aa	42.1	4.2
OambLPAAT2	<i>Oophila amblystomatis_SRR5445907</i>	SRR5445907	359 aa	41.5	2.74
PmorLPAAT2	<i>Pandorina morum_SRR13719246</i>	SRR13719246	325 aa	41.2	2
EdLPAAT2	<i>Edaphochlamys debaryana_SRR13719274</i>	SRR13719274	324 aa	40.6	1.8
VaulLPAAT2	<i>Vitreochlamys aulata_SRR13719273</i>	SRR13719273	325 aa	40.0	4.6
DsLPAAT2	<i>Dunaliella salina</i>	KAF5838861.1	333 aa	40.0	2.35

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
DsalLPAAT2	<i>Dunaliella salina_SRR9129444</i>	SRR9129444	335 aa	39.4	2.06
CuwoLPAAT2	<i>Chlamydomonas sp.UWO_241_SRR13696614</i>	SRR13696614	342 aa	39.4	3.65
RsLPAAT2	<i>Raphidocelis subcapitata</i>	GBF93205.1	380 aa	39.4	1.58
Ce LPAAT2	<i>Chlamydomonas eustigma</i>	GAX78532.1	253 aa	38.8	1.59
MyarcLPAAT2	<i>Microglena sp.YARC_SRR18294403</i>	SRR18294403	362 aa	38.5	2.87
SceLPAAT2	<i>Scenedesmus sp. NREL 46B-D3</i>	KAF6259536.1	358 aa	37.1	1.64
McLPAAT2	<i>Micractinium conductrix</i>	PSC72189.1	426 aa	36.8	1.55
CnivLPAAT2	<i>Chlamydomonas nivalis_SRR12517273</i>	SRR12517273	360 aa	36.2	1.59
VcLPAAT2	<i>Volvox carteri_98847</i>	XP_002957774.1	303 aa	35.9	1.58
ChvuLPAAT2	<i>Chlorella vulgaris</i>	KAI3438340.1	360 aa	35.0	1.63
ChdeLPAAT2	<i>Chlorella desiccata</i>	KAH7617329.1	372 aa	34.7	1.71
ApLPAAT2	<i>Auxenochlorella protothecoides</i>	XP_011401335.1	385 aa	34.7	1.58
ChsoLPAAT2	<i>Chlorella sorokiniana</i>	PRW57325.1	363 aa	33.8	1.53
ChvaLPAAT2	<i>Chlorella variabilis</i>	XP_005852243.1	357 aa	33.2	1.58
ChprLPAAT2	<i>Chloropicon primus</i>	QDZ21586.1	335 aa	28.8	1.62
BpraLPAAT2	<i>Bathycoccus prasinos_ERR2041002</i>	ERR2041002	334 aa	27.9	1.66
OILPAAT2	<i>Ostreococcus lucimarinus</i>	XP_001421357.1	286 aa	25.3	1.63
VrLPAAT2	<i>Volvox reticuliferus</i>	GIM16764.1	156 aa	25.0	1.69
PproLPAAT2	<i>Pycnococcus provasolii_ERR2041000</i>	ERR2041000	326 aa	23.2	1.64
CosLPAAT2	<i>Coccomyxa subellipsoidea</i>	XP_005647638.1	197 aa	22.9	1.63
NoLPAT4	<i>Nannochloropsis oceanica</i>	BAW99690.1	445 aa	16.2	1.55
CrLPAAT1	<i>Chlamydomonas reinhardtii</i>	XP_042921325.1	332 aa	12.9	1.59
CrLPAAT1-ΔCST*	<i>Chlamydomonas reinhardtii</i>	XP_042921325.1	302 aa	12.9	1.69
AGPAT1	<i>Homo sapiens</i>	NP_001358366.1	283 aa	12.6	3.6
NoLPAT3	<i>Nannochloropsis oceanica</i>	BAW99691.1	482 aa	12.1	1.6

Gene name	Organism	GenBank ID/Accession Number	Protein size	Identity (%)	TAG profile m/z (577/603) OP:OO
EcplsC	<i>Escherichia coli</i>	HBN6599230.1	245 aa	10.9	1.59
BnBAT2-ΔCST*	<i>Brassica napus</i>	NP_001302928.1	258 aa	10.3	1.9
SI11848	<i>Synechocystis sp. PCC6803</i>	WP_010873224.1	225 aa	10.3	1.6
BnBAT2	<i>Brassica napus</i>	NP_001302928.1	344 aa	10.3	1.57

[0367] **Table 112** shows the amino acid sequences of LPAATs described herein.

[0368] In some embodiments, a microalgal cell provided herein comprises an exogenous gene that encodes for an LPAAT in **Table 112**. In some embodiments, the exogenous gene can comprise a sequence that encodes for an enzyme with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a LPAAT in **Table 112**. In some embodiments, the exogenous gene can comprise a sequence that encodes for an enzyme with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 83-106, 155, and 156.

Table 112: LPAAT Amino Acid Sequences

SEQ ID NO:	LPAAT	Amino Acid Sequence
83	VcomLPAAT2	MSLISKALSLPSFLLSVFVFYWSLPIFAIPYRIRFANVGKR NDMLDWAKALFAFFRVTVLKQGEQGLYRGGPCLYLCN HRSWADFFVDVYLTEGRAALMSRWLVFFVFPVFCTSC ILRGIVLFKRGITLDKEAFNAWLDTLRRSTVPGLLIYPE GHRSLKPTSLPLKRGMLHFAYSRRLPVQLIVTRGKEDIL SEKTMAMKYGRTLVTYTSKVLKPTDYDSFDAFMGDVQ TSWDAGWKASYEAPTKGVPQLSMAGAHEYDYPATMR WQQLAITLFSIVVFAAVLYGTWWGLGAVLAAAGPLGQ QVVVLAATSWLGASLIRSCR
84	VitrLPAAT2	MSLISKASSLPSFLFSVFVFYWSLPIFAVPYRIRFANMGK RNDMLDWAKALVAFFRVTVLKQGEQGLYRGGTCLYL CNHRSWADFFIDVYLTEGRAALMSRWLVFFVFPVFCTS CLILRGIVLFKRGITLDKEAFNAWLDTLRRSIVPGLVV YPEGHRSLKPTSLPLKRGMLHFAFGRQLPVQLIVTRGKE

SEQ ID NO:	LPAAT	Amino Acid Sequence
		DVLSEKTMAMKYGRTLVTSSYKVLKPSDYASFDAMT DIQTCWDSCWRAAYEAPTKGVPQLSMVGAHEYDYPRT IWWQQLWITLASILIFAGVLYGTWRGLASLLAAATTSG ALGPQVLVTVAAGSSLLRSLL
85	CchaLPAAT2	MSIVTKVLNLP SFLFSVFV FYWSLPLF AILYRIRFANVGK RNDMLDWAKTLVGFFHVTILKQGELSLYRDGPCLYLC NHRSWADFFIDTYLTEGRAALMSRWLVYFVFPVFCTSV IILRGIVLFKRGSIADKEAFNAWL DATLRKSIVPALLVYP EGHRS LRPTSLPLKRGMLHYAYSRRLPVQLIVTSGKEA VMSEK TMSVRFGR TLVT TYSKVLR PADYPTFD AFFSDI QATWDGCWDAAYKQPSAGAPQLRLVDPHGYNYPARM WWLQLGV TLLSIGVMAAVIAASWWGLGALLAATGPA KQVV LGVVVSLLGASLLRSCL
86	PjapLPAAT2	MSVIYKALS LPSFLFSVFV FYWSLPLF ASVYRIRFANFGK RNDMLDWAKALVAFFRVTVLKQGEMSLYRGGPCLYL CNHRSWADFFIDTYLTEGRAALMSRWLVYFVFPVFCTS VLILRGIVLFKRGHIVDKEAFNAWL DATLFKSTVPGLLV YPEGHRS LKPA SLPLKRGMLHFAFSRKLPVQVICTRGKE EVLCEK TLSARWGR TLVVTFSKVIK PSEYDSFDAFFNDV QSTWDACWQLSYHQPTAGVPELSMKDAHEYDYPRSM WWLQLGITLLSFILAAVLWGSWVAVRAGFATLGSVQQ AVVLGTAGWIGASMVCGFL
87	VbolLPAAT2	MSALKKALS LPSFLFSVFV FYWSLPIFASVYRIRFARAG KRNDMLDWATSLVAFFRVTVLKQGQFSIYRGGPCILYLC NHRSWADFFIDMYLTEGRAAFMSRWMVFFVFPVFCSA VLILKG IILFKRGRILDKEAFNAWL DATLAKSTIPGLVVY PEGHRS LKPTSLPLKRGMLHYAFSRKLPVQLIVTRGKEE VLSEK TLSMRFGR TLVT SYSKVMKPSDYGSFDAFMTDV QSGWDQCWKEAYSAPTHGLPQLSMAGATEYDYPASM RWQH LAMTLFSIVVFAGVLAASWWGVGALLSAAGPTG KQVV VLLASCWFGASLLRSFL

SEQ ID NO:	LPAAT	Amino Acid Sequence
88	PmorLPAAT2	MSLVTKFLSLPSFLFSVFIFYWSLPIFAIPYRIRFANVGKR NDMLDWAKALVGFFRVTVLKPGATSVYRGGPCLYLCN HRSWADFFIDVYLTQGQSALMSRWLVFFVFPVFCTSLI LRGIVLFKRGITILDKEAFNAWLDATRARSIVPGLVVYPE GHRSLKPTSLPLKRGMIHYAFSRRLPVQLIVTSGKEHVL SEKTMSVKFGKTLVTSYSKPLKPSDYATFDAFMADIQS NWDQCWKEAYTAPARGMPEVSMAGAHSYAYPRLTW CLQLAITVVSIVILGGVLAGSWRGAAAVLAAAGPVGQQ VLVSLALWAGVSLLRSFL
89	VcarfLPAAT2	MSITSKALSLPSFLFTVFVFYWSLPLFAIIRIRFANVGKR NDMLDWAKALVAFFRVTVLKQGEMSLYRGGRCCLYLC NHRSWADFFIDTYLTEGRAALMSRWMVFFAFPVFSTSV LILRGIVLFKRGHIADKEAFNSWLDATLRKSTVPALLVY PEGHRSKPRSLPLKRGMLHFAFSRQLAVQMVITRGKE EVLSEKTCSARWGRTLVTYYSKVIKPTDYDSFDAFFNEI QTTWDACWQLSYHQPTGVPQLSLKGAHEYDYPVNM WWLQLGVTLTSITILVAVLYGSWVALRAGVSSLGSSIT QQAVVLGVAGWLGVSLRSFL
90	EcylLPAAT2	MSLISKALSLPSFLFSVFVFYWSLPFFATTYRIRFANVGK RNDMLDWAKTLVAFFRVTVLKQGNMSLYRGGRCCLYL CNHRSWADFFIDTYLTEGRAALMSRWLVYFVFPVFCTS VIILRGIVLFKRGHIADKEAFNAWLDATFSRSIVPGLLVY PEGHRSKRTSLPLKRGMMHYAYSRRIPVQLIVTRGKE EVLSEKTL SARWGRTLVTYYSKVLKPSDYDSFDAFFNDI QAQWDACWKTSYDTPAAGVPQLSMVDAHEYDYPRRM WWLQLGMTLLSIVILAAVLWGSWLGIRAGLATLGS LQ QQAVVWGASGWL GASLLLSFF
91	GmulLPAAT2	MGAAGKLLSLPSFLFSVFVFYWSLPIFALVYRIRFASSGR RNDMLDWAKSIVRFFGVTVVKVGENSELYREGPCLYLC NHRSWADFFIDAYLTEGRAALMSRWLVFFVFPVFCTSC LILRGIVLFKRGTIADKEGFNRWLD SKLGSSHVPGLLVY PEGHRSRPSLPLKRGMLHYAYGRKLAVQIICTAGKE

SEQ ID NO:	LPAAT	Amino Acid Sequence
		GVMSEKTMVHFGRITLVTTFSQVLRPSEYPTFDAFFAA VQSAWDASWKAANQTPSAGLPRLSMEGAQDYDYPPA MRAQQLGVTLLSFVVFGGALLVTWRLLAALLAAAGAA QQAVALLLAGWTGVSMRLSFL
92	GvirLPAAT2	MGAASKLLGLPSFLFSVFVFWSLPIFAIVYRIRFASPGR RNDMLDWAKSIVGFFGVAVHKAGTNSLYRDGPCLYLC NHRSWADFFIDAYLTEGRAALMSRWLVFFVFPVFTSC LILRGIVLFKRGTIADKEAFNSWLDATLGRSIVPGLLVYP EGHRSLRPASLPLKRGMLHYAFGRKLPVQVVCTAGKE GVMSEKTMAVRFGRRLLVTNFSQVIRPADFPFDFAFSAA VQSAWDGCWKAAYETPAAGLPRLSMEGSHEYEDYP APMRVLQVCITLLSIVLFLGVLLGSWRALAAALAAAGA AQQVVVALLATWLGASMLRSCL
93	VferLPAAT2	MSFISKAVGLPSFLFSVFVFWSLPIFALTYRIRFASKGK RNDMLEWAKALVRFFGVKVLQGEHSLYRGGPCLYLC NHRSWADFFIDAYLTEGRAALMSRWLVYFVFPVFTSV MVLRGIVLFKRGTIADKEAFNAWLDAKMKSSTIPGLLV YPEGHRSLKRTSLPLKRGMLHFAFSRGLPVQLVMCAGK EEVLSEKRMVFRGRTLVATYSEVLKPGNYPTFDAFFS GIQTAWDSCWQAAYKQPPEGAAPLPMVNTTEYDYP MRRLQLIVTLLSIVVLA AVLAGSVLVTVAAAAVGPMP AGVVVAVLASWLGGSMRLSFL
94	VaulLPAAT2	MGAASKVLGLPSFLFSVFVLYWSLPIFAVLIRIRFAKMI GKRNDVLDWAKSLVRFFGITVLRQGGQEQLYTGGPCLY LCNHRSWADFFLDVYLTEGHAAVMSRWLVYFVFPVFC TSCLILKTIVLFKRGTIRDKEGFNRWLDAVLARSNPGL LVFPEGHRSLRPASLPLKRGMLHYAYGRKLAIQMVVTR GKEEVMSEKTMVFRGRTLVTTYSKVLRPADYPDFEAF FADLQSTWDGCWEAAYRKPREDAVRHSLGAAPGFHYP FATRVAQLGATLGSIALFGLVLVGCWRGLAAALAATG AAQQAVAALLAVWVGSMRLSFL

SEQ ID NO:	LPAAT	Amino Acid Sequence
95	Ch_CCAC2762_LPAAT2	MRQLKSLIQLPYFLFCVVFYWSFPIGCLTHHHFLKFSG KRNDMHTWSETMCSFFGIRFVKYGEKLSKGSRNILYL SNHRSWADFFVDVYLTEGNAAMLSRMLVFFVFPIFMTS VVVLKGILFKRGTIADKEKFNAWLDVMLERSPIPGMLV YPEGHRSTRPHSLPLKRGMLYYAHSRGMPVQIIISNGKE GVMSEKQRRAGFGKTVVVGFSVIDTAAYKGNVEGFM ARVQEEWNKLWGQVYSADPAGLPPLRQDPPMLDYAA SMRTQQLAIAVFSMFALAGVLWVTWRMFAWLTALAG PLQHALAVLLLTWFAASIIAGVMGPSLPTHSSSLQNPPS LKADKSVSAAAAAAAAASGDGSLHEAPSGKAPAAAAT QNHNHQH
96	DsalLPAAT2	MGQSTLQKLISLPTFFLSIFLMYWSLPLCTLCGYLRLLDF KSTRKDMYNWCRFMTRLFRIKVKKYGSKSLRKDAGRH ILYLCNHRSWTDFFLDAYLTEGNASLSRWLVFAVFPV FIVSVIVLRGVILFKRGTILDKEKFNRWLDKILSTPIPGV LVYPEGHRSLADKSLPLKRGMLYYAHSRKFPVQIIISRN KEDVLSEKTMKAHFGATIITGCSDLIESDGKDIKVLSEI QAAWDDLWEKVYAADATALPPLEQSNIFEFDYSMGMR VKQLLVCCSSVLVFLAAMVVFGKLSCLIIYAAMGDTER KVLVTLILYVWFFVSMLNSRAPSATQ
97	MyarcLPAAT2	MSAEKLLHAPSFVITCLVLYWSLPLSVLFGYIRAFQWTG PRNDMYSWGQFMSDFFGVKFVRVGNEKLYKPKKGAN VIYLCNHRSWADFFCDVYLTQGNAALLSRMLVFFIFPFF MSSVVILKGVVLFKRGKVVDKDAFNKRLDGKLADSPIP GLLVYPEGTRSLKDLPLKRGMLYFAHSRNIPVQIVVS SGKEYVLTEKKIRARIGRTVVSTFSAKITPSDFPSCEAFL EHVQKEWDRLWPAVFTADADKAPVLVNEDIPMHDYA NTMRWAQLGMAVLSTTVATIMFLLSWKVVDLIFGIFGP LKQVLYMLLFTWVSLSLVASFLGPKHLPKGVLPSPVLP LPVARPPPPSQEPSSSGSK
98	CuwoLPAAT2	MAANMATFPFFLLTFFFLYWSMAIGTLVGRVKFWQYN GTNRNEMFVWAKLCSDFLGVKYLKTGSGSLYKKERCV

SEQ ID NO:	LPAAT	Amino Acid Sequence
		YLSNHRSWADFFVDVYLTEGIAQAMARTMVGIAFPMFI GAATILRGILLFKRGSIA NKDKFNTWLDDKLNSSPCSSL LVFPEGHRSTKSTSLPLKRGMLYYAHSRGLPVQVIMTR GKELVLSEKHHHAGYGTQLITGFSELVLSKDKEFEQFW TEVQAMWDRMWA EVYSVDPSTAVPYTQDVPGSVYPL SMSVLYTVFTVSTVIFLLSTLWIMARIFGMISPLPAMLQ MVVWGAVPVT LAVSFRASIIDNAKTSSSAVIKTAKKAQ
99	CmoeLPAAT2	MASFVDLLLQFPYFGLTIFFFYWSPLASIVYRIRFASAG PRKDIYEWTTAMVQFLGVRIRHQGKQTL YKKERC VYL CNHRSWADFFVDLH LTQGLAAPMSRTMVFFAFPV FMSI CIVLKG VLLFKRG TIADKTKFNKWLDDSLGNSPV SCLL VYPEGHRSTKAKSLPLKRGMLYYAHSRGLPVQIIHTAG KEAVLSEKRM RASYGQTLVTGFSQPIQSKDFKDFAFS AEVQRSWDALWTEVYSANPSALPPLVQEDVPTADYPPS MRTGMLLVSLAAIALFLAFVALLAYITGPVMSLFPAPLR VPLILFKLSWHAASFLYCRAKDTPGKV KAA
100	OambLPAAT2	MALARSSSSSALLLWLLRLPYFLLTITFFYWSATIGALIY RVRFLSTGPRNDMYDWTL LMTSFLGLHFHQLGAQRLH CAPRVVFLANHRSWADFFVDIHLTQGT AAPMSRALVAF AFPVFMCTAWLLRGLIIFKRG AIEDKKKFNSWLDEQLD ASAGSNLLVYPEGHRSTRPTSLPLKRGMLYYAHSRGLP VQIIMTAGKESVLSEKHQCASFGQHLLTSFSPVISSKDK DFEVFATEVQQRWDSLWREVFSTSTQGVPLLHQASVPL FDYPPAVRTHMALASSAFILMLLLAAGAAVLAVRATLA LMPPVLQVVVLAASSLLMLASFLTSAAITSTTTTTTTTT RQPQAVADKGSKES
101	EdLPAAT2 (<i>Edaphochlamys debaryana_SRR</i> 13719274)	MSALNKALGLPSFLFSVFVFWSLPIFATFYRIRFASNG KRNDVLDWAKSLVRFFGVTVLQQGEHSLYRGGACLYL CNHRSWADFFIDAYLTQGLAALMSRWLVYFVPVFCTS CMILRGVVLFKRGTIADKDAFNKWLDDTLGSSPVPGLL VYPEGHR SIKPTSLPLKRGMLHYAYS RKKPVQIIITRGK DEILSEKTLAVHFGRTVVTTF SKVLRPADYATFEAFFTE

SEQ ID NO:	LPAAT	Amino Acid Sequence
		LQSTWDSCWVAAYTTPIAGIPRLSMADPHAYDYSSTMR AQQLAITLFSILVLVGVLGASWRVWTAVLAATGTAQTV VVLVLASWVGTSMLRSFL
102	VsteLPAAT2	MSFLGKALSFPALFSVFVFYWSLPIFASVYRIRFVKRG KRNDMLDWAKSLVDFFRVRVLKQGENSLYRGGRCLYL CNHRSWADFFLDMYLTEGRAAFMSRWMVFFVFPVFCS ACLVLRGIVFFKRGIIIRDKEAFNAWL DATLSRSIVPGLV VYPEGHRSLRPTSLPLKRGMLHFAFDRRLPVQVIVTSGK EGVLSEKTLAMRFGCTLVVSYSKVLKPSEYDTFEAFMS DVQAQWDTCWKAVYGSPTTGAPELSMVG AHQYDYPL SMRVQHVAMTLLSIGIMGAVVIGSWRGWNAVLLALGG PVVQQAVVLLGA AWLGVSLLRSL
103	GoctLPAAT2	MTTMREEKWHTQQADVGAKSTASKAERVGATSKLLG FPSFLFSVFVLYWSLPIFAILYQIRFASPGRNNSLDWAK SLVGFFGVTVVKTGEDSLYRGGPCLYLCNHRSWADFFI DAYLTEGRAALMSRWLVFFVFPVFLTAVRIIRGVVLFK RGAVADKEAFNAWL DATLKRSVVPGLLVYPEGHRSLR PTSLPLKRGMLHFAYGRKL PVQIIVTAGKESVMSEKAL AVHFGRALVTKFSRALRPTDYPGFDAFFAAVQSTWDEC WKAAYGTPAAGTPRLTIDGAHG DY PPLMRWQLLVVT LLCILLFGGVLLASWRALAALLAAGAARRAVVLLLAL WVGASASRSFV
104	CnivLPAAT2	MARGSGSIGRRPGWLSLLLDLPAFLLAIFILYWSSALGA ALGYIRFTKMRGLRDDTFEWSKFMLHAFVRIVRLGPV LPYRQSPCMYLCNHRSWADFFVD TYCTEGRSALMSRW LVWWVFPVFMSVCRIIGGVVLFKRGAVFDKEKFNAWI DKRLTEVPGHSLLVFPEGHRSSSSSLVLRGMLHYAHS RKLPVQVVL SAGKEGV LSETRQCVRFGR TVVV TYADVI KSGDFPNFDDFLATVQSTWEEKWRQVHSAPKDGLPAL DPVGMSFEYTP TTRWRHVAATLSSYVFMAVVLYASWQ AAAYALLAMGPLAPVISTILVWWSAVSLTLAWLPERTP SARHWAAPATNGPVASSPVD

SEQ ID NO:	LPAAT	Amino Acid Sequence
105	PproLPAAT2	MSLVTEILLRITFCVDLFLQIVIPLLALLYSPRDAGTHN MSAWTSKMLGYRIHKAGQNTCYVPEEKSTRVMYLAN HRSWGDFIVDRLLTGFRGGSLSRIMVIVGIPMPATLAWL GSGQRGTSALWFFKRAKGATPALWATLDRNFEVAIEK AVVVYPEGHRNRSDKPLPIRTGTLRYAFDRNVHVQVLI AEGKERVINEAQLVTRRSPNVVTVSFSDPVPPKNYSTFE GFHGAVCEAWRTTWLAAYGAAAEAGYCAPPALGPD GMAESATAADHKRIHRPPAAVPWTPPNPGPPPNQHASL GMHMARLCIVGAISAAAYTYE
106	BpraLPAAT2	MGLSSPSKKLESPSSSRDKYVDTRSLAEKGVHLIALLI AF PILAFSYPLSLLTRGTLHGWNFSKEIRPPITQIWADFMT SNVMNVRFKIVSQQLYRDGKCVYLCNHRADFFID MSLCEGRSFTLSRMLVMYVFPVFMVPAVFGAVFPFRR DKGGGHEELNNSIDKHWSRFEQYFPGFLCYPEGTRNIKP HSLPLKRGMLRYAHSRKL PVQIIICAGKEKVL SQKRFAA ERNHVL PVA YSSVIHSDSY PVFEDFAE AIRHSWADEWA NAYNHENKMEKLKDFKPSERTIRYERWVDFTTGT VVS FFVFCALVANVLQIIFPKIHELGA
155	CtLPAAT1- ΔCST*	MARKSSLAQAAPVAPSPSAKPELPARSALVCHAAAASV PLPNSDSAPQPNVLLAKIRAIMFFAWSFLLSLPLFVTMM VMAPLVLAFDKYRRLAQHFVNNLWACASTAPFYKV TII GRENLPPPDKPVVYVANHQSFLDIYSLFHLQRPFKFISKT SNFLIPIIGWSMFLTGHVMINRVDRRSQ LKCLQQCRDLL AEGAPVLFFPEGTRSLDCKMAGFKKGAFSVA AKAGVE VVPITLLGTGSLMPSGKESQLRPGQVTIVVHKALPPNKN ADQLCDAARQAVASSLPPELVGSATEMAPDEQ
156	BnBAT2-ΔCST*	MSDLSGAATPESTYPEPEIKLSSRLRGICFCLVAGISAIVL IVLMIIGHPFVLLFDRYRRKFHHFIAKLWASISIYPFYKT DIQGLENLPSSTPCVYVSNHQSFLDIYTLLSLGQSYKFI SKTGIFVIPVIGWAMSMGVVPLKRMDPRSQVDCLKR CMELVKKGASVFFFPEGTRSKDGR LGPFKKGAFTIAAK

SEQ ID NO:	LPAAT	Amino Acid Sequence
		TGVPVVPITLMGTGKIMPTGSEGILNHGDVRVIIHKPIYG SKADVLCEEARNKIAESMNLIS

[0369] **Table 113** shows conserved LPAAT motifs and the percent identity (%) of the most active LPAATs across the entire LPAAT protein relative to PedLPAAT2. The transmembrane domains of these LPAATs were compared and the schematic diagrams of the LPAATs are shown in **FIG. 11**. The LPAAT catalytic domain NHX₄D is shown in a shaded bar, binding motif EGTR or EGHR is shown in a black bar, and transmembrane domains are indicated by a gray bar.

Table 113: Percent identity against PedLPAAT2 and LPAAT motifs

Gene name	Percent identity (%)	LPAAT Motifs			
		I (NHX ₄ D)	II ((F/Y)XXR)	III (EGXR)	IV (Proline)
PedLPAAT2	100	NHRSWAD (SEQ ID NO: 107)	GIVLFKRG (SEQ ID NO: 110)	LLLYPEGTRNT KP (SEQ ID NO: 120)	PFQIIVT ^N NKE (SEQ ID NO: 135)
VcomLPAAT2	45.882	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLIYPEGHRSL KP (SEQ ID NO: 121)	PVQLIVTRGK E (SEQ ID NO: 136)
CmoelLPAAT2	45.882	NHRSWAD (SEQ ID NO: 108)	GVLLFKR G (SEQ ID NO: 112)	LLVYPEGHRST K (SEQ ID NO: 122)	PVQIIHTAGK E (SEQ ID NO: 137)
CchaLPAAT2	43.824	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL R (SEQ ID NO: 123)	PVQLIVTSGK E (SEQ ID NO: 138)
VferLPAAT2	43.529	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL K (SEQ ID NO: 124)	PVQLVMCAG KE (SEQ ID NO: 139)
GvirLPAAT2	43.529	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL R (SEQ ID NO: 123)	PVQVVCTAG KE (SEQ ID NO: 140)
VcarfLPAAT2	43.529	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL K (SEQ ID NO: 124)	AVQMVITRG KE (SEQ ID NO: 141)
CrLPAAT2	43.235	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRST K (SEQ ID NO: 122)	PVQIVVTRGK D (SEQ ID NO: 142)

Gene name	Percent identity (%)	LPAAT Motifs			
		I (NHX ₄ D)	II ((F/Y)XXR)	III (EGXR)	IV (Proline)
VbolLPAAT2	43.235	NHRSWAD (SEQ ID NO: 108)	GIILFKRG (SEQ ID NO: 113)	LVVYPEGHRSL K (SEQ ID NO: 125)	PVQLIVTRGK E (SEQ ID NO: 136)
AgLPAAT2	42.941	NHRSWAD (SEQ ID NO: 108)	GVIFIDRK (SEQ ID NO: 114)	LLVYPEGHRSL K (SEQ ID NO: 124)	PVQIIITRGKE (SEQ ID NO: 143)
CiLPAAT2	42.941	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRST K (SEQ ID NO: 122)	PVQVIITRGK D (SEQ ID NO: 144)
VitrLPAAT2	42.941	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LVVYPEGHRSL K (SEQ ID NO: 125)	PVQLIVTRGK E (SEQ ID NO: 136)
Ch_CCAC2762_L PAAT2	42.941	NHRSWAD (SEQ ID NO: 108)	GIILFKRG (SEQ ID NO: 113)	MLVYPEGHRSL TR (SEQ ID NO: 126)	PVQIIISNGKE (SEQ ID NO: 145)
EdLPAAT2	42.647	NHRSWAD (SEQ ID NO: 108)	GIILFKRG (SEQ ID NO: 113)	MLVYPEGHRSL K (SEQ ID NO: 127)	PVQVIITRGK D (SEQ ID NO: 144)
EcyLPAAT2	42.353	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL K (SEQ ID NO: 124)	PVQLIVTRGK E (SEQ ID NO: 136)
PjapLPAAT2	42.353	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL K (SEQ ID NO: 124)	LPVQVICTRG KE (SEQ ID NO: 146)
GmulLPAAT2	42.059	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVYPEGHRSL R (SEQ ID NO: 123)	AVQIICTAGK E (SEQ ID NO: 147)
OambLPAAT2	41.471	NHRSWAD (SEQ ID NO: 108)	GLIIFKRG (SEQ ID NO: 115)	LLVYPEGHRST R (SEQ ID NO: 128)	PVQIIIMTAGK E (SEQ ID NO: 148)
PmorLPAAT2	41.176	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LVVYPEGHRSL K (SEQ ID NO: 125)	LPVQLIVTSG KE (SEQ ID NO: 149)
VaulLPAAT2	40	NHRSWAD (SEQ ID NO: 108)	GIVLFKRG (SEQ ID NO: 111)	LLVFPEGHRSL R (SEQ ID NO: 129)	AIQMVVTRG KE (SEQ ID NO: 150)

Gene name	Percent identity (%)	LPAAT Motifs			
		I (NHX ₄ D)	II ((F/Y)XXR)	III (EGXR)	IV (Proline)
DsLPAAT2	40	NHRSWTD (SEQ ID NO: 109)	GVILFKR (SEQ ID NO: 116)	MLVYPEGHRSLA (SEQ ID NO: 130)	PVQIIISRNKE (SEQ ID NO: 151)
DsaLPAAT2	39.412	NHRSWTD (SEQ ID NO: 109)	GIVLFKRG (SEQ ID NO: 111)	VLVYPEGHRSLA (SEQ ID NO: 131)	PVQIIISRNKE (SEQ ID NO: 151)
CuwoLPAAT2	39.412	NHRSWAD (SEQ ID NO: 108)	GILLFKRG (SEQ ID NO: 117)	LLVFPEGHRSTK (SEQ ID NO: 132)	PVQVIMTRGKE (SEQ ID NO: 152)
MyarcLPAAT2	38.529	NHRSWAD (SEQ ID NO: 108)	GVVLFKR G (SEQ ID NO: 118)	LLVYPEGTRSLK (SEQ ID NO: 133)	PVQIVVSSGKE (SEQ ID NO: 153)
AGPAT1	12.647	NHRSWAD (SEQ ID NO: 108)	GIVLFKR (SEQ ID NO: 119)	WVFPEGTRNH N (SEQ ID NO: 134)	PIVPIVMSSY (SEQ ID NO: 154)

Example 53 Composition of the OPO Oil

[0370] The average fatty acid content for eight representative lots of high *sn*-2 palmitic acid *Prototheca moriformis* algal oil derived from the strain and methodology described in Example 32 above, is provided in Table 114.

Table 114: Average Fatty Acid Composition of the TAG of high *sn*-2 palmitic acid *Prototheca moriformis* algal oil

Fatty Acid	Wt.% of Total Fatty Acids
C16:0	31.5
C18:0	2.6
C18:1 n-9	55.2
C18:2 n-6	6.3
Other FA	4.4
Total	99.4

[0371] Oleic acid is mainly esterified to the external positions (*sn* 1/3) of the TAGs (Table 115) while the middle position (*sn*-2) is mainly occupied by palmitic acid (Table 116). Approximately 70% of total palmitic acid is esterified in the *sn*-2 position. TAG analysis on eight OPO algal oil lots confirms that TAGs with oleic acid esterified at the *sn*-1 and *sn*-3 positions and palmitic acid esterified at the *sn*-2 position (i.e., 1,3-dioleoyl-2-palmitoyl-glycerol or OPO) represent more than 40% of TAG species in OPO algal oil of the present disclosure.

Table 115: Fatty Acid Profile of the *sn*-1/3 Positions in High *sn*-2 Palmitic Acid *Prototheca moriformis* Algal Oil

Fatty Acid	% of Total Fatty Acids
C16:0	3.3
C18:0	4.7
C18:1 n-9	80.3
C18:2 n-6	8.4
Other FA	3.3

Table 116: Fatty Acid Profile of the *sn*-2 Position in High *sn*-2 Palmitic Acid *Prototheca moriformis* Algal Oil

Fatty Acid	% of Total Fatty Acids
C16:0	69.3
C18:0	0.5
C18:1 n-9	22.8
C18:2 n-6	3.7
Other FA	3.7

Example 54 Comparison of the Composition of Oil Mix Formulated with OPO Oil

[0372] OPO algal oil can be used as a human milk fat substitute intended to be used as an at least partial replacement for vegetable oils in conventional infant formulas for infants born at term up to a level of, for example, 60% of the total lipid composition of the formula. Examples of oil blends for infant formulas comprised of OPO algal oil in combination with other vegetable oils are reported in Table 117.

Table 117: Examples of oil mixes formulated with OPO algal oil in combination with conventional vegetable oils to be used in infant formula. All values in g of oil per 100 g of oil mix

Oil	Oil Mix 1	Oil Mix 2	Oil Mix 3
OPO algal oil	50	55	60
Sunflower oil	22	22	22
Canola oil	19	19	18
Coconut oil	9	4	0
Total	100	100	100

[0373] The combinations of the algae oil with vegetable oils as exemplified in Table 117, provide a fatty acid composition that is primarily enriched with oleic, palmitic, linoleic, lauric, myristic, capric, and α -linolenic acids are shown in Table 118.

Table 118: Fatty acid composition of the oil mixes formulated with OPO algal oil in combination with conventional vegetable oils to be used in infant formula.

Fatty acid profile (g per 100g of fatty acids)	Oil Mix 1	Oil Mix 2	Oil Mix 3
C8:0	0.7	0.3	0.0
C10:0	0.5	0.2	0.0
C12:0	4.2	1.9	0.0

Fatty acid profile (g per 100g of fatty acids)	Oil Mix 1	Oil Mix 2	Oil Mix 3
C14:0	1.7	0.8	0.0
C16:0	18.8	19.9	21.2
C18:0	2.6	2.6	2.7
C18:1 n-9	47.1	49.5	52.7
C18:2 n-6	18.7	19.0	19.4
C18:3 n-3	1.7	1.7	1.8
Other fatty acids	3.8	4.0	2.2

[0374] When compared to the fatty acid composition of human breast milk (Table 119), the content of total palmitic acid is in the range of the palmitic acid observed in human milk. The data set displayed in Table 119 has been computed from a series of original studies representing a total of 835 samples of mature human milk collected in 10 countries described below:

- Yuhas et al. 2006: Australia (n=48), Canada (n=48, Chile (n=50), Japan (n=51), Mexico (n=46), Philippines (n=54), UK (n=44) and USA (n=49)
- Thakkar et al. 2013: Singapore (n=50)
- Giuffrida et al. 2016: China (n=345)

Table 119: Fatty acid composition of human milk samples (N=835) collected in several countries (Yuhas et al., 2006; Thakkar et al., 2013 and Giuffrida et al., 2016)

Fatty acid profile (g per 100g of fatty acids)	Average	Min	Max
C8:0	0.2	0.2	0.3
C10:0	1.7	1.4	2.4
C12:0	6.0	4.2	13.8
C14:0	6.1	3.6	12.1
C16:0	20.6	18.6	23.3
C18:0	5.8	4.8	6.8
C18:1 n-9	31.4	21.9	36.5
C18:2 n-6	14.1	7.9	22.8
C18:3 n-3	1.2	0.4	2.0
Other fatty acids	0.2	0.2	0.3

Example 55 Levels of Palmitic Acid in Microalgal Oil

[0375] The combinations of the algae oil with vegetable oils as exemplified in Table 117 above provide high levels of palmitic acid esterified in *sn*-2 position as shown in Table 120. The level of *sn*-2 palmitic acid reported in human milk is in the range of $67.8 \pm 4.6\%$ as reported in the literature (Giuffrida et al., 2019). The level of *sn*-2 palmitic acid provided by the algae OPO oil used in combination with other vegetable oils is in the range of the level observed in human milk (Table 116).

Table 120: Level of palmitic acid in *sn*-2 position in oil mixes formulated with OPO algal oil in combination with conventional vegetable oils to be used in infant formula.

Fatty acid	Oil Mix 1	Oil Mix 2	Oil Mix 3
Palmitic acid (PA) in <i>sn</i> -2 position (% of total PA)	63	65	66

[0376] Table 120 shows the fatty acid profile of (1) the total TAGs, (2) *sn*-1(3) positions and (3) *sn*-2 position of (a) the parent *Prototheca moriformis* microalgae strain, (b) the high palmitic acid strain generated from the parent strain by mutagenesis and (c) the OPO strain engineered by insertion of an LPAAT gene is illustrated in Table 121. Data is expressed in grams per 100 grams of total fatty acids.

Table 121: Comparison of the fatty acid profile of the parent strain (CHK22), high-palmitic acid (CHK100) strain, and the OPO engineered strain (Example 32).

Fatty acid	CHK22 (Parent strain)			CHK100			OPO engineered strain		
	TAG	<i>sn</i> -1(3)	<i>sn</i> -2	TAG	<i>sn</i> -1(3)	<i>sn</i> -2	TAG	<i>sn</i> -1(3)	<i>sn</i> -2
C16:0	25.3	36.5	3	30.3	35	6	31.5	3.3	69.3*
C18:0	3.3	4.7	0.5	2.3	4.7	0.5	2.6	4.7	0.5
C18:1 n-9	61.1	48.9	85.6	57.4	50.8	81.6	55.2	80.3	22.8
C18:2 n-6	6.9	6.1	8.4	6.3	6	8.5	6.3	8.4	3.7
Other FA	3.4	3.9	2.5	3.8	3.5	3.3	4.4	3.3	3.7

*When expressed in g per 100 g of total palmitic acid, the level of palmitic acid esterified in the internal (*sn*-2) position is 73.3%.

[0377] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. An algal oil comprising triglycerides (TAGs), wherein:
 - a. a total TAG content of the algal oil has a fatty acid profile comprising about 25 wt. % to about 35 wt. % of C16:0 fatty acid; about 1.5 wt. % to about 3.0 wt. % C18:0 fatty acid; about 48 wt. % to about 58 wt. % of C18:1 n-9 fatty acid; about 4.5 wt. % to about 7.5 wt. % of C18:2 n-6 fatty acid; and about 2 wt. % to about 6 wt. % of other fatty acids;
 - b. a *sn*-1(3) position of a TAG has a fatty acid profile comprising about 1.5 wt. % to about 4.5 wt. % of C16:0 fatty acid; about 1.5 wt. % to about 4.5 wt. % of C18:0 fatty acid; about 70 wt. % to about 90 wt. % of C18:1 n-9 fatty acids; about 5.5 wt. % to about 9 wt. % 18:2 n-6 fatty acids, and about 1.5 wt. % to about 4 wt. % of other fatty acids; and
 - c. a *sn*-2 position of a TAG has a fatty acid profile comprising about 65 wt. % to about 75 wt. % of C16:0 fatty acid; about 0.1 wt. % to 1 wt. % of C18:0 fatty acid; about 18 wt. % to and 25 wt. % of C18:1 n-9 fatty acids; about 1 wt. % to about 5 wt. % 18:2 n-6 fatty acids; and about 1 wt. % to about 5 wt. % of other fatty acids;wherein the algal oil is derived from a modified microalgae strain.
2. The algal oil of claim 1, wherein the modified microalgae strain is a genetically modified microalgal strain.
3. The algal oil of claim 1, wherein the modified microalgae strain is derived from classical strain improvement.
4. The algal oil of claim 1, wherein the modified microalgae strain is derived from both classical strain improvement and genetic modification.
5. The algal oil of any of the above claims, wherein the algal oil is produced from a *Prototheca* cell.
6. The algal oil of any of the above claims, wherein the algal oil is non-naturally occurring.
7. A composition comprising the algal oil of any one of claims 1-6, and one or more excipients.
8. The composition of claim 7, wherein the composition is a nutritional supplement for humans or animals.

9. The composition of claim 7, wherein the composition is an infant formula.
10. The composition of claim 9, wherein the infant formula comprises one or more of whey, casein, lactose, vitamin D, human milk oligosaccharides (HMOs), and antibodies.
11. The composition of claim 9, wherein the infant formula further comprises plant proteins.
12. An oil blend comprising:
 - a. about 50-60 g algal oil per 100 g of the oil blend, wherein the algal oil comprises TAGs,
wherein at least about 60% of fatty acids in an *sn*-2 position of the TAGs is a C16:0 fatty acid;
 - b. about 20-30 g sunflower oil per 100 g of the oil blend; and
 - c. about 15-25 g of canola oil per 100 g of the oil blend.
13. The oil blend of claim 12 further comprising at least about 3-10 g of coconut oil per 100 g of the oil blend.
14. The oil blend of claim 12 wherein the algal oil is the algal oil of any one of claims 1-6.
15. A composition comprising the oil blend of any one of claims 12-14, and one or more excipients.
16. The composition of claim 15, wherein the composition is a nutritional supplement for humans or animals.
17. The composition of claim 15, wherein the composition is an infant formula.
18. The composition of claim 15, wherein the infant formula comprises one or more of whey, casein, lactose, vitamin D, human milk oligosaccharides (HMOs), and antibodies.